

**Origins of the human malaria parasites
P. falciparum and *P. vivax***

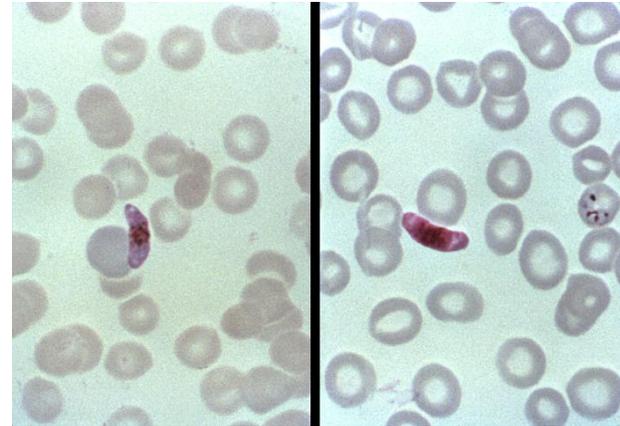
Origins of *P. falciparum* and *P. vivax*

- ◆ Introduction
- ◆ *Plasmodium* infections in African apes
- ◆ Origin of *Plasmodium falciparum*
- ◆ Origin of *Plasmodium vivax*
- ◆ Likelihood of additional *Plasmodium* zoonoses.
- ◆ Age of human malaria

What causes malaria?

Parasite

- Single cell eukaryotic parasite of the *Plasmodium* genus
- Five *Plasmodium* parasite species that cause malaria in humans:
- ***P. falciparum*, *P. vivax***, *P. ovale*, *P. malariae*, *P. knowlesi*
- Species is important - both the disease and the treatment are species dependent

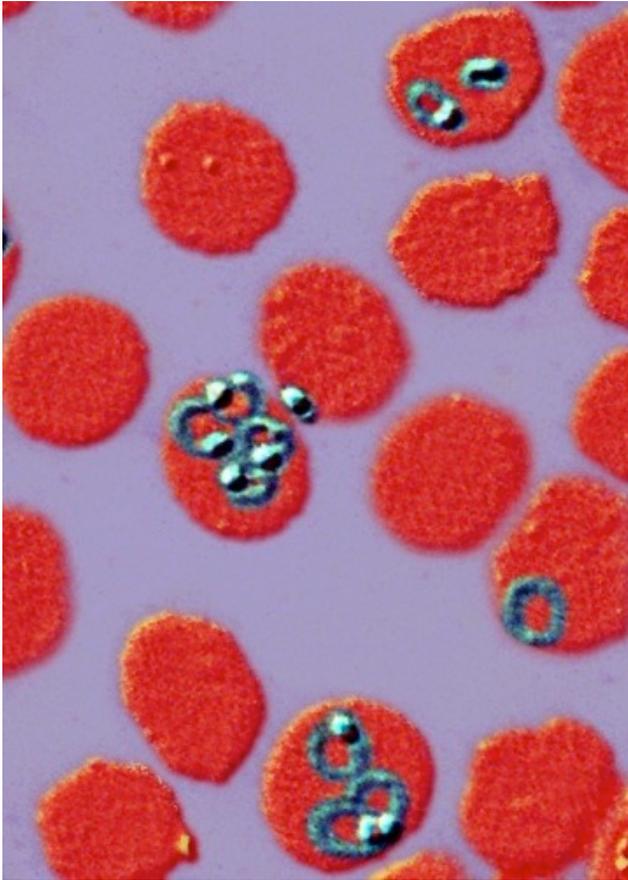


Vector

- Mosquitoes of the *Anopheles* genus
- Parasite must cycle through both the mosquito and vertebrate host to complete life cycle



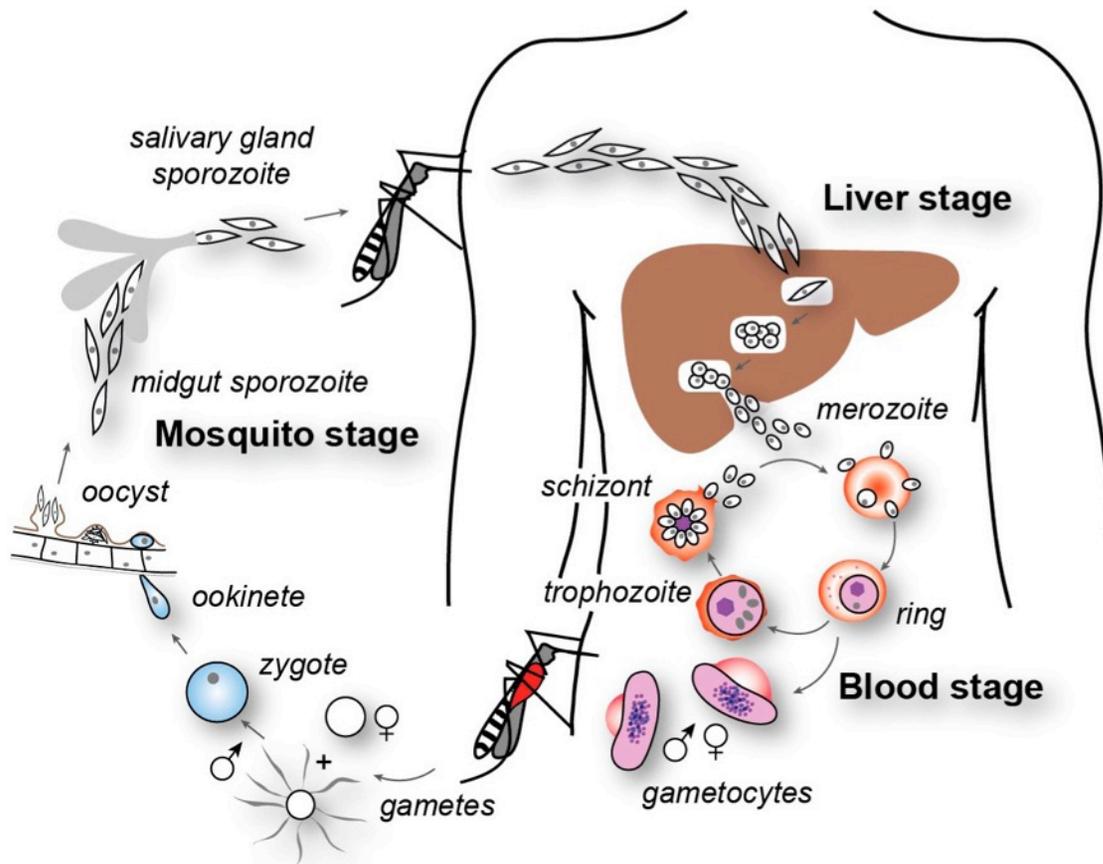
Size matters?



- *P. falciparum* genome has 14 chromosomes, 23Mb (23,000,000 base pairs),
- 5,300+ genes predicted, 80.6% AT content.
- Humans: 2,900 Mb (100x), 30,000 genes
- Bacteria: *Y. pestis* (plague) 5.4Mb, *M. tuberculosis* (TB) 4.4Mb (malaria has 4-5x size)
- HIV-1: 10,000 bp, nine genes
- What does that extra genome size buy you?
- For starters, a complex life cycle....

Image: CDC/ Steven Glenn, Laboratory & Consultation Division

Life cycle of *Plasmodium falciparum*



Malaria – Today

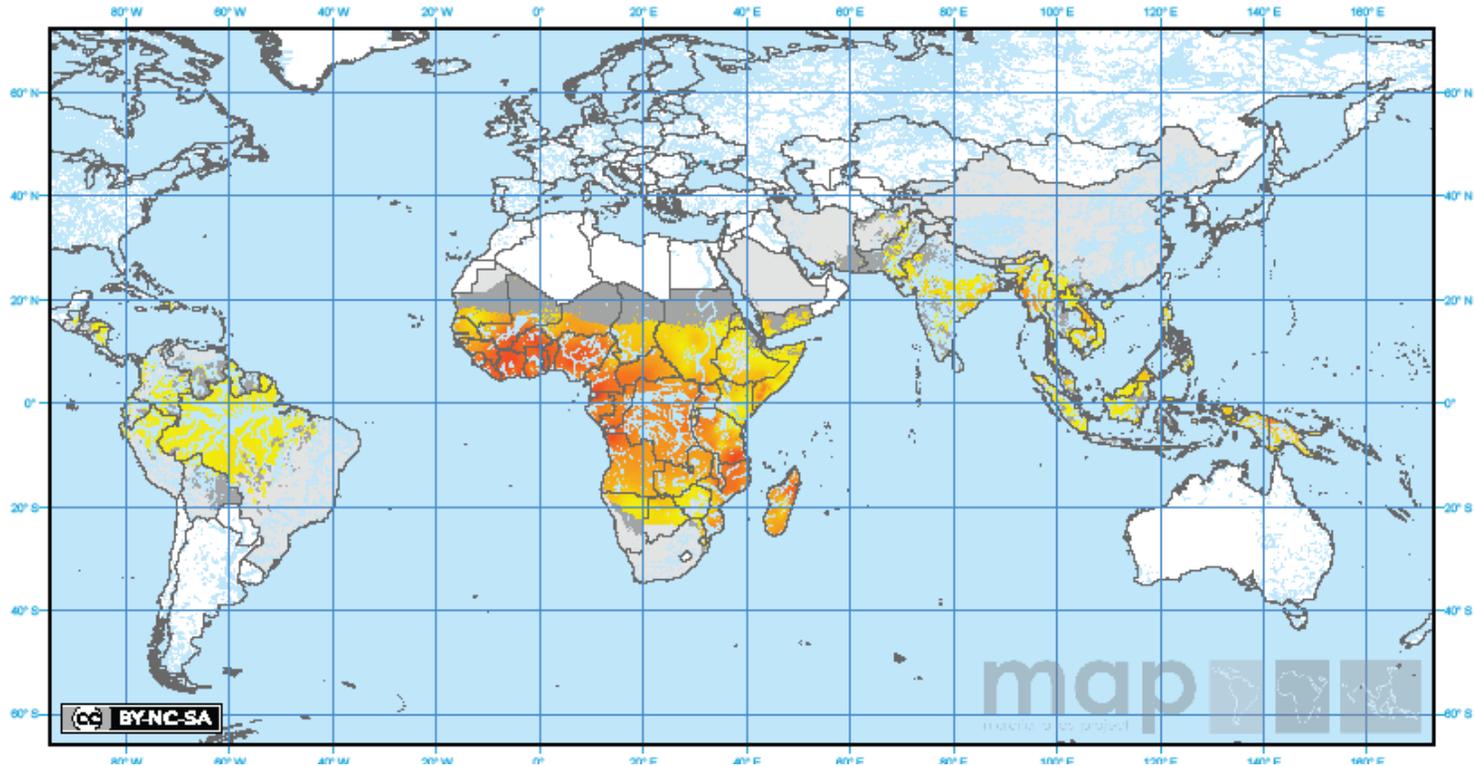
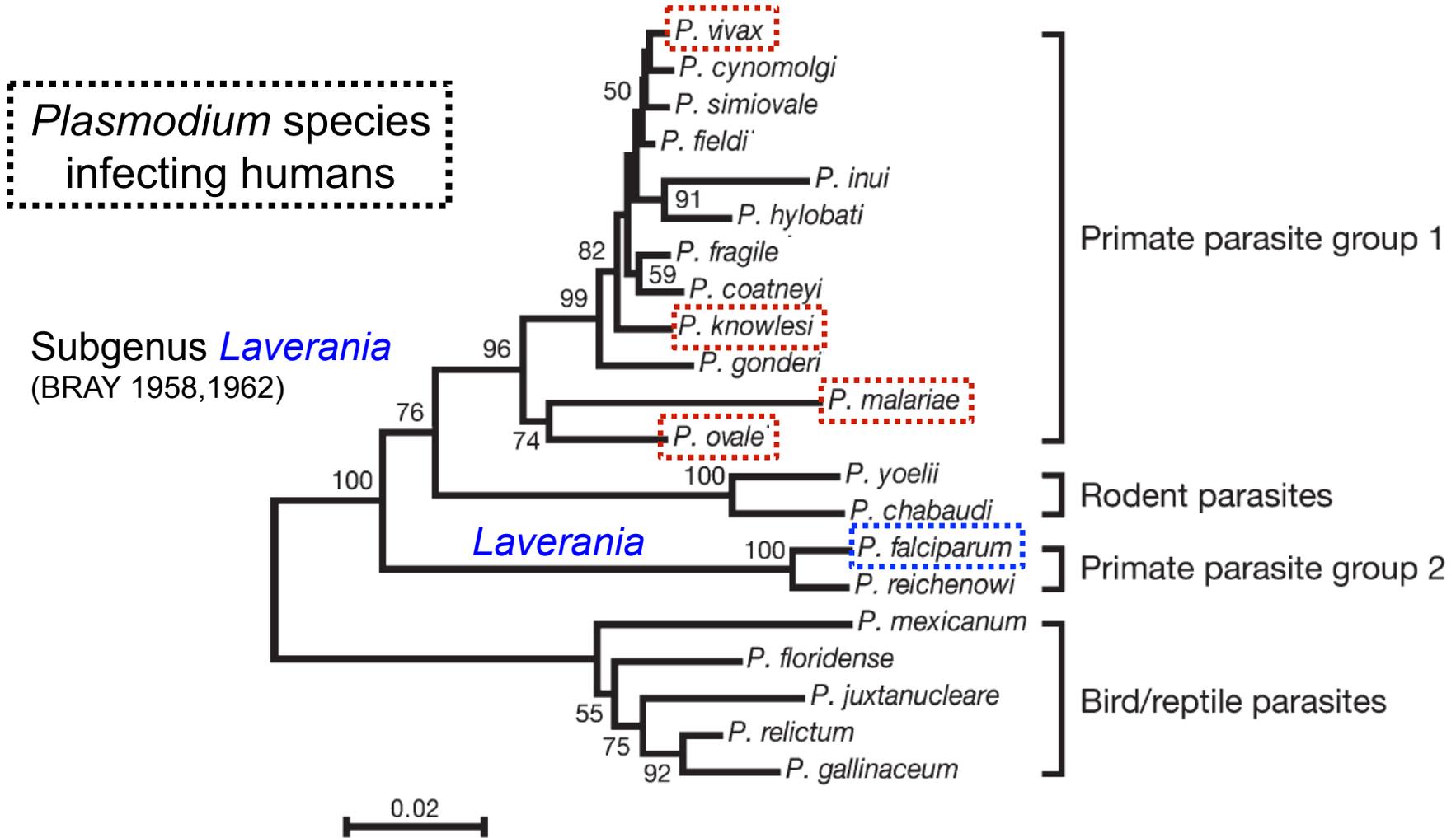


Image licensed to the Malaria Atlas Project (MAP; www.map.ox.ac.uk) by Hay, S.I. et al. (2009). A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Medicine* 6(3): e1000048.

- ~40% of the world's population at risk
- 300 - 500 million new infections every year
- >1 million deaths, largely in children in Africa
- No vaccine
- Widespread drug resistance

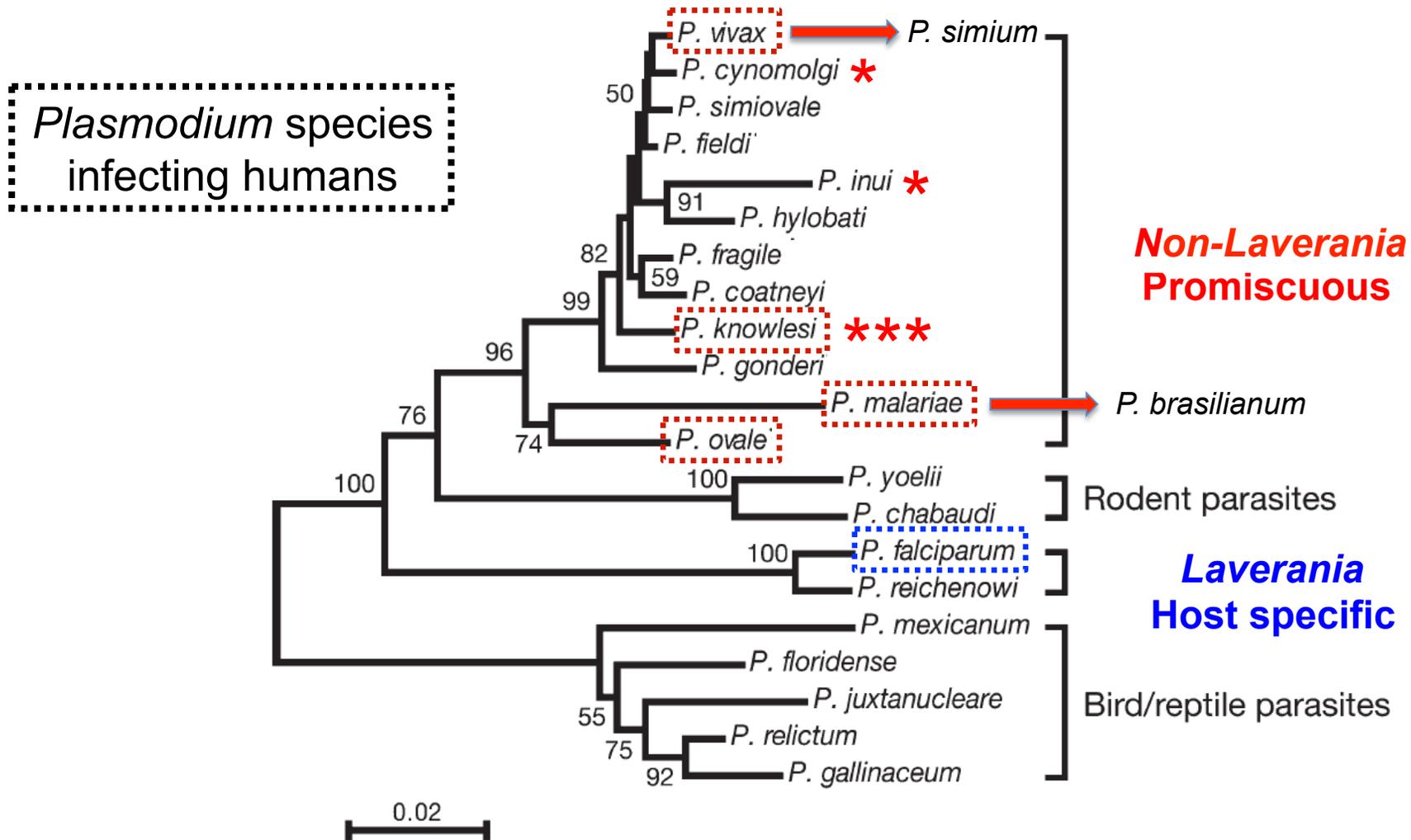
Phylogeny of Malaria Parasites



Plasmodium Species Naturally Infecting Primates

Region	Parasite	Date	Host
Asia	<i>P. pitheci</i>	1905	Orangutan
	<i>P. inui</i>	1905	Macaque (can infect humans)
	<i>P. cynomolgi</i>	1905	Macaque (can infect humans)
	<i>P. eylesi</i>	1965	Gibbon
	<i>P. hylobati</i>	1939	Gibbon
	<i>P. jefferyi</i>	1964	Gibbon
	<i>P. youngi</i>	1964	Gibbon
	<i>P. fieldi</i>	1962	Macaque
	<i>P. simiovale</i>	1965	Macaque
	<i>P. coatneyi</i>	1965	Macaque
	<i>P. fragile</i>	1965	Macaque
	<i>P. knowlesi</i>	1932	Macaque (zoonotic infection)
America	<i>P. brasilianum</i>	1908	Multiple New World monkey species (reverse zoonosis of <i>P. malariae</i>)
	<i>P. simium</i>	1951	Howler monkeys and Spider monkeys (reverse zoonosis of <i>P. vivax</i>)
Africa	<i>P. gonderi</i>	1908	Mangabeys/Mandrills
	<i>P. schwetzi</i> *	1920	African apes
	<i>P. rhodaini</i> *	1939	African apes
	<i>P. reichenowi</i>	1922	Chimpanzee
Madagascar	<i>P. lemuris</i>	1963	Lemur
	<i>P. girardi</i>	1952	Lemur

Host Specificity of Malaria Parasites



Phylogeny from: HAYAKAWA *et al.* (2008) *Mol.Biol.Evol.*25:2233

Origins of Human *Plasmodial* Parasites?

Plasmodium falciparum

- co-evolved with human ancestors since (at least) 7 Myr ago
- spread out-of-Africa
 - greater genetic diversity in Africa

Plasmodium vivax

- cross-species transmission from Asian monkeys (1-2 Myr ago?)
- spread towards Africa
 - greater genetic diversity in Asia



4 February 1999

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nature

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Chimpanzees and HIV-1

Volcanic eruptions Exploding under strain

Embryology Female development in mammals

Clathrate hydrates Structural complexities

Non-Invasive Detection of SIV

Collect fecal samples (in RNA_{later})
at field sites throughout Africa.

Screen for virus specific antibodies:
ECL Western blot

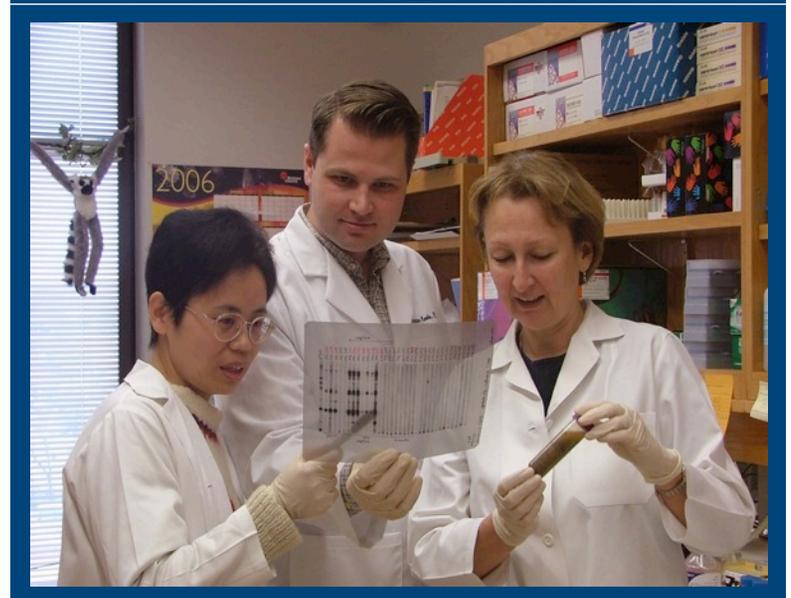
Extract **fecal nucleic acids**:

Viral RNA:

- partial and full-length genomes
- phylogenetic analyses
- construction of infectious clones

Host DNA:

- species/subspecies origin
(mitochondrial DNA)
- enumeration of individuals
(microsatellites; gender)



African great apes are natural hosts of multiple related malaria species, including *Plasmodium falciparum*

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Contributed by Francisco J. Ayala, December 14, 2009 (sent for review October 27, 2009)

Non-invasive detection of *Plasmodium* sequences in ape fecal DNA

What might we find in apes?

Ape *Plasmodium* species:

Parasites from chimpanzees and gorillas

Eduard Reichenow in Cameroon ~1917

Saul Adler, Donald Blacklock in Sierra Leone ~1922

three morphologically distinct species:

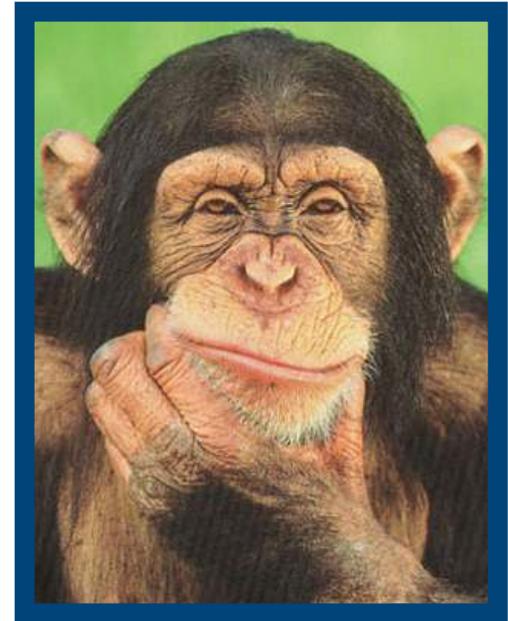
Human parasite

P. falciparum
P. malariae
P. vivax or *P. ovale*

Ape parasite

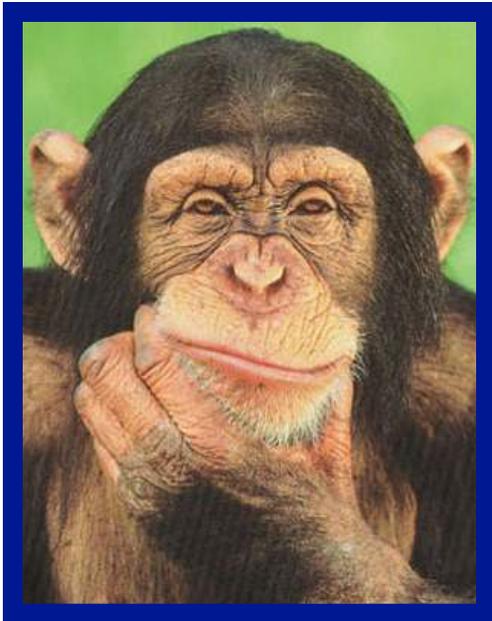
P. reichenowi
P. rhodaini
P. schwetzi

ONE isolate in culture
no samples
no samples



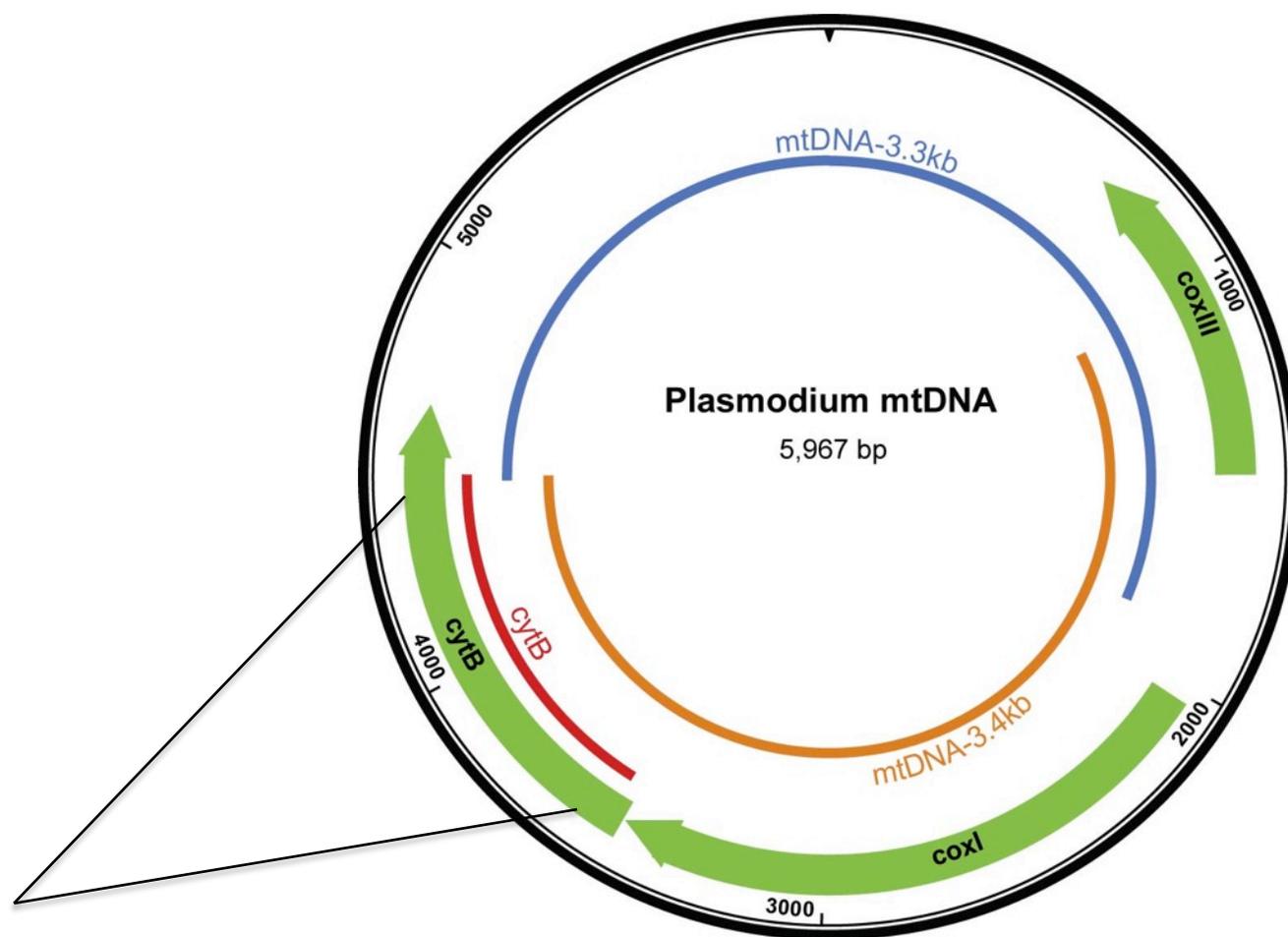
Plasmodium infections of wild apes

Questions:



- ◆ Which ape species and subspecies are infected with *Plasmodium* in the wild?
- ◆ How prevalent is ape malaria?
- ◆ How many different *Plasmodium* species are circulating in the wild?
- ◆ Are the human parasites of ape origin?

Screening for *Laverania* parasites in wild apes



**Diagnostic PCR:
Amplify 950 bp *cytB* fragment**

Prevalence of Ape *Laverania* Infections

Chimpanzees

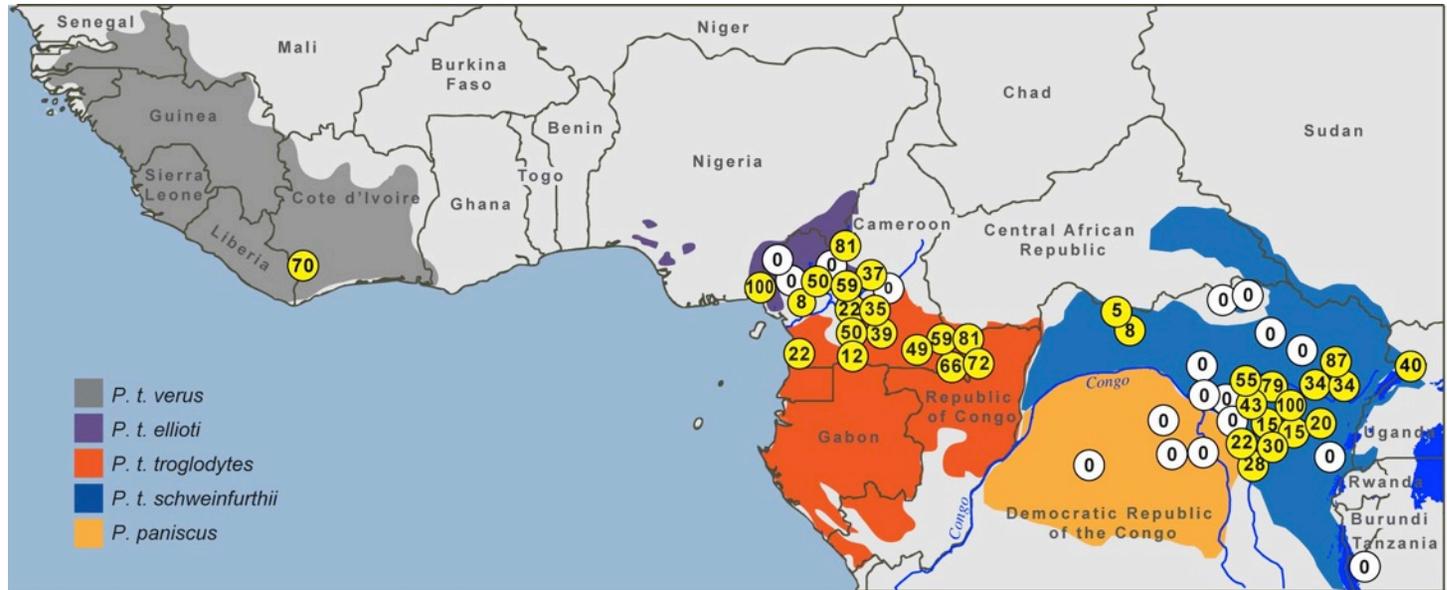
n=46

48% prevalence

Western Gorillas

n=20

37% prevalence



Eastern Gorillas

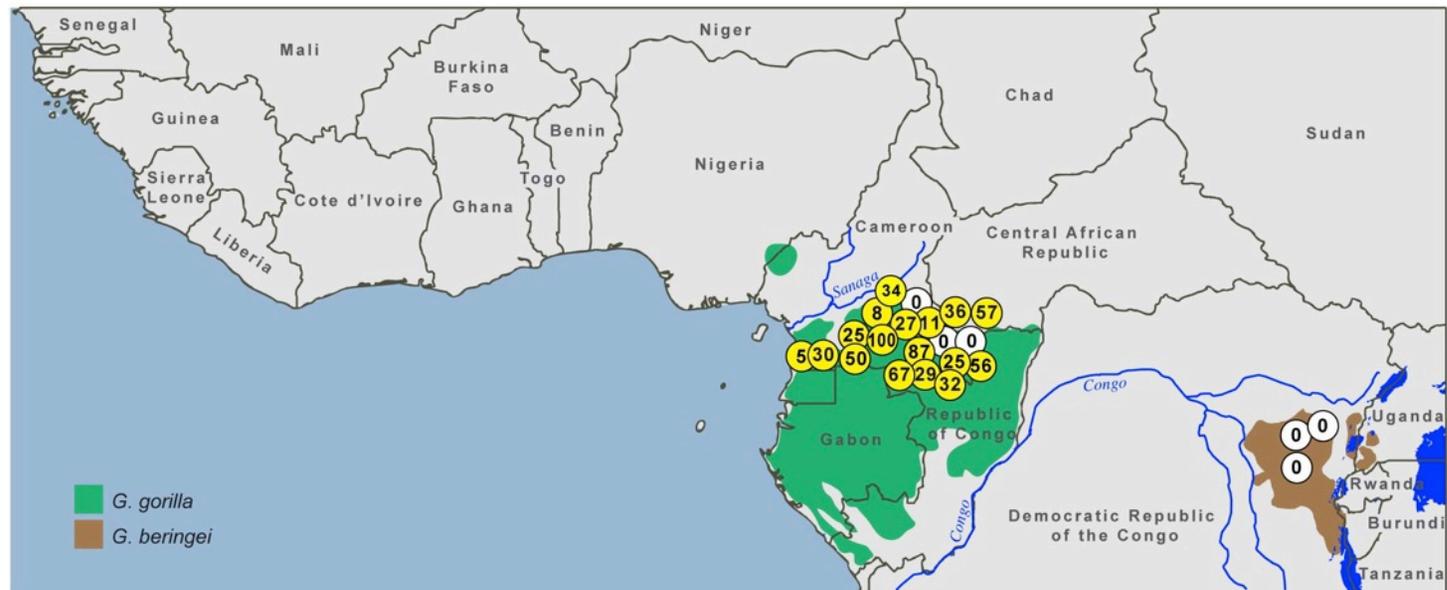
n=3

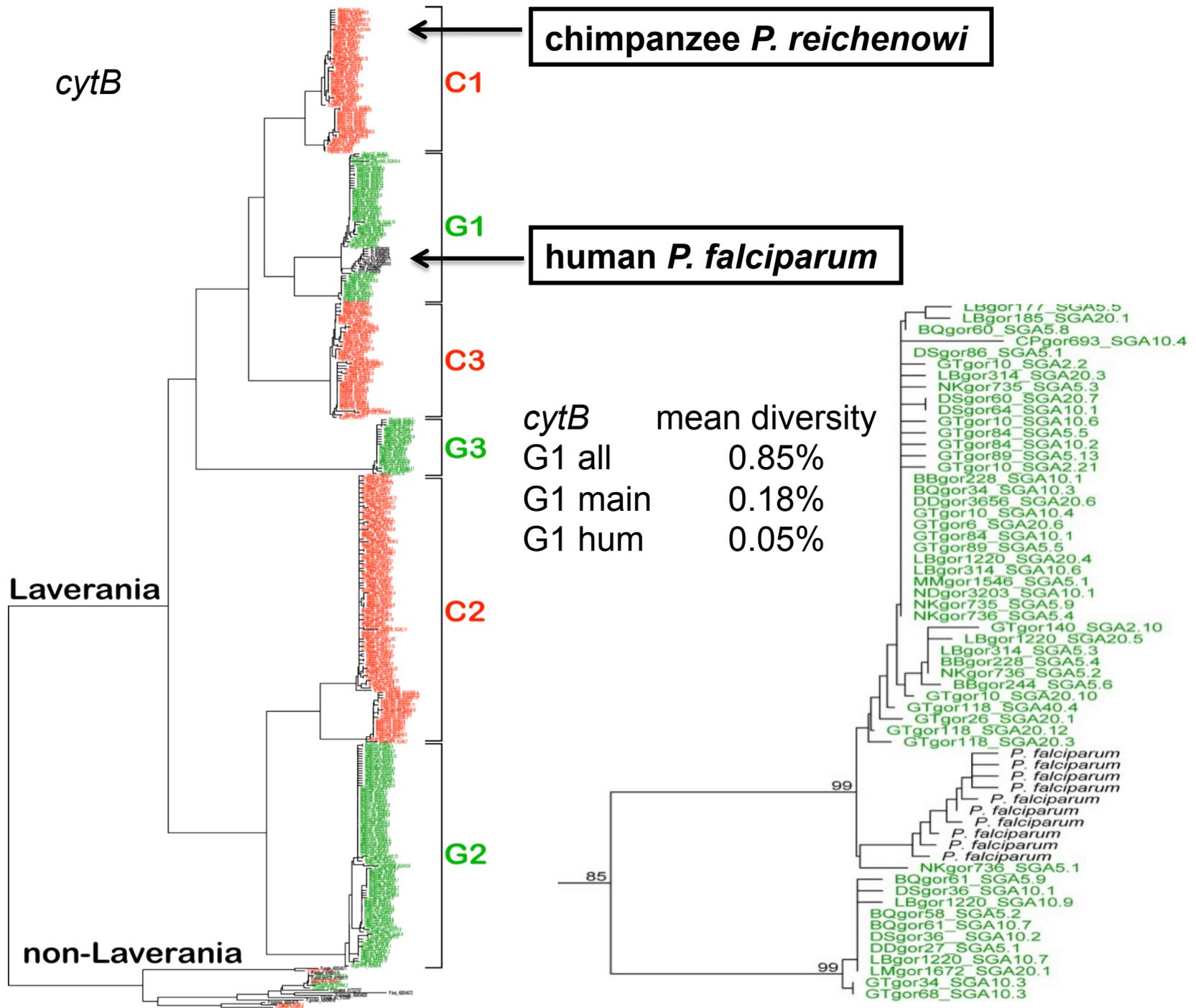
146/0

Bonobos

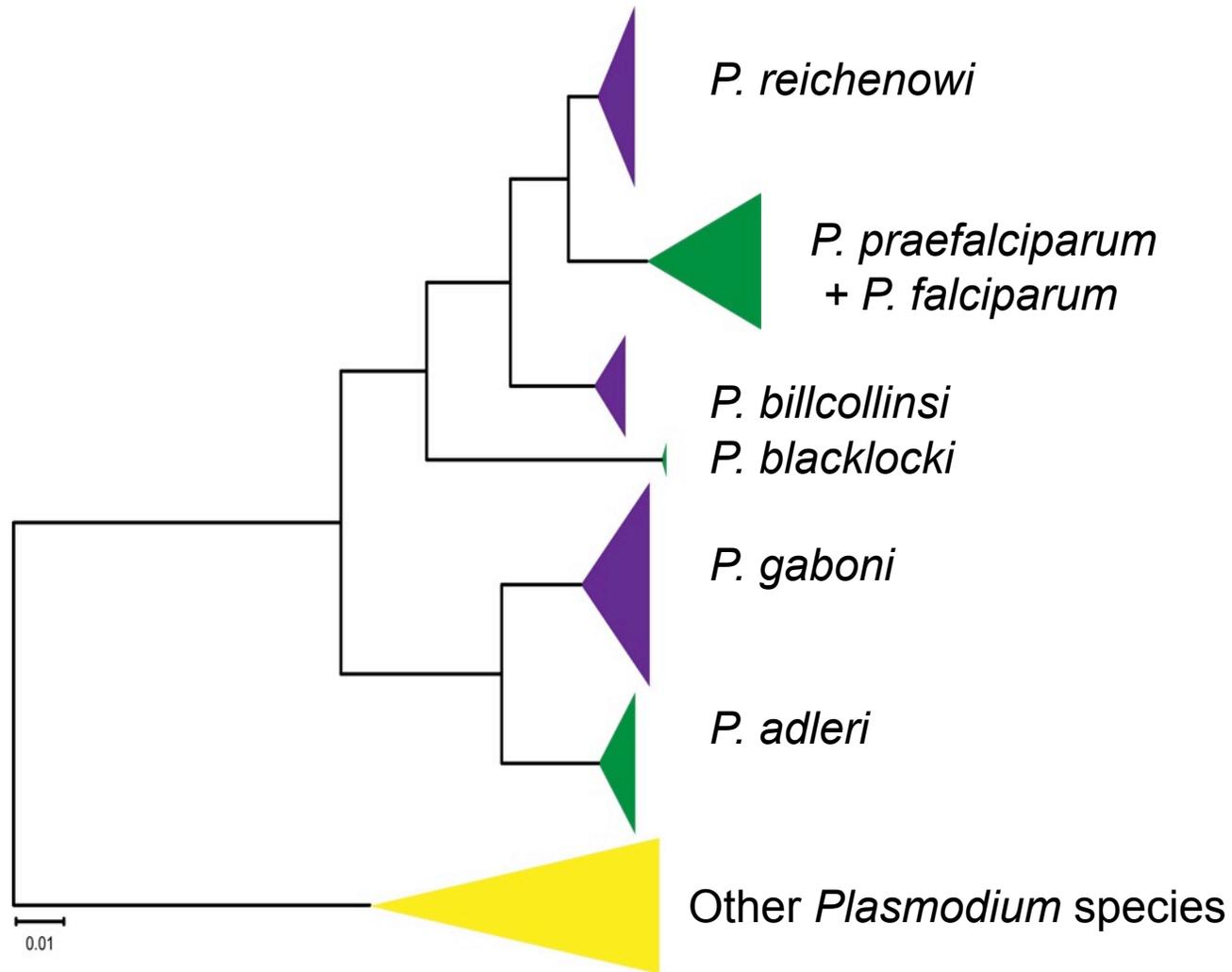
n=5

210/0





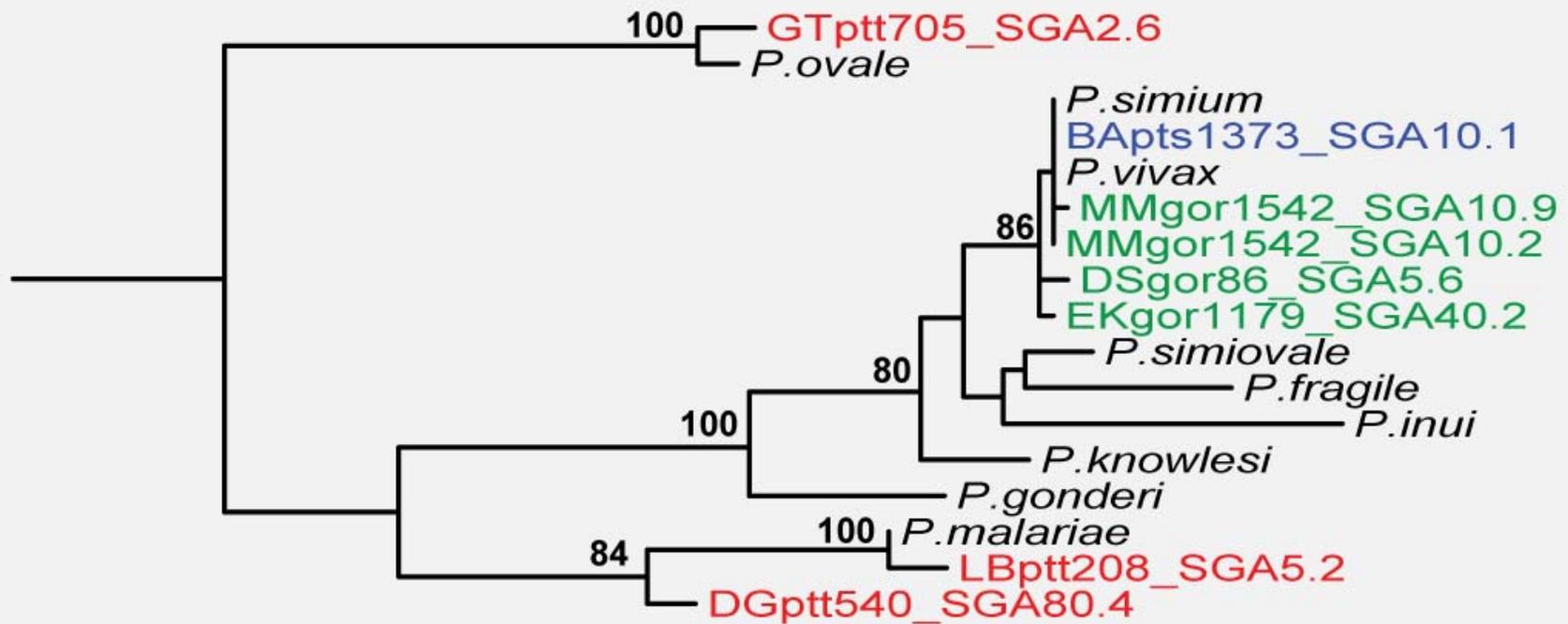
African Apes Harbor Six *Laverania* Species



Take Home Points

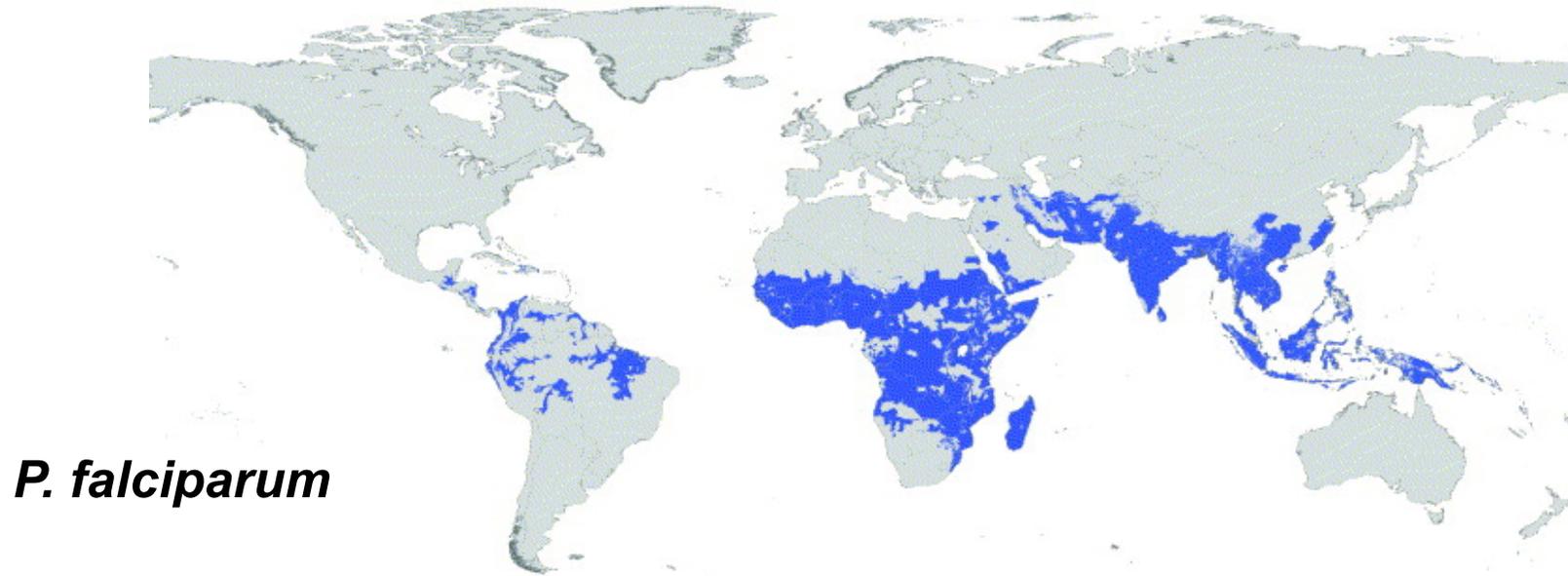
- Chimpanzees and western gorillas are each naturally infected with three *Laverania* species.
- Ape *Plasmodium* infections are highly prevalent, widely distributed, almost always comprised of mixed parasite infections, and remarkably host species-specific.
- Humans acquired *P. falciparum* following a single gorilla-to-human transmission event.
- *P. falciparum* parasites found in captive bonobos and chimpanzees are of very recent human origin.
- There is no evidence of human *P. falciparum* infection in wild-living apes or monkeys.

Other *Plasmodium* Species

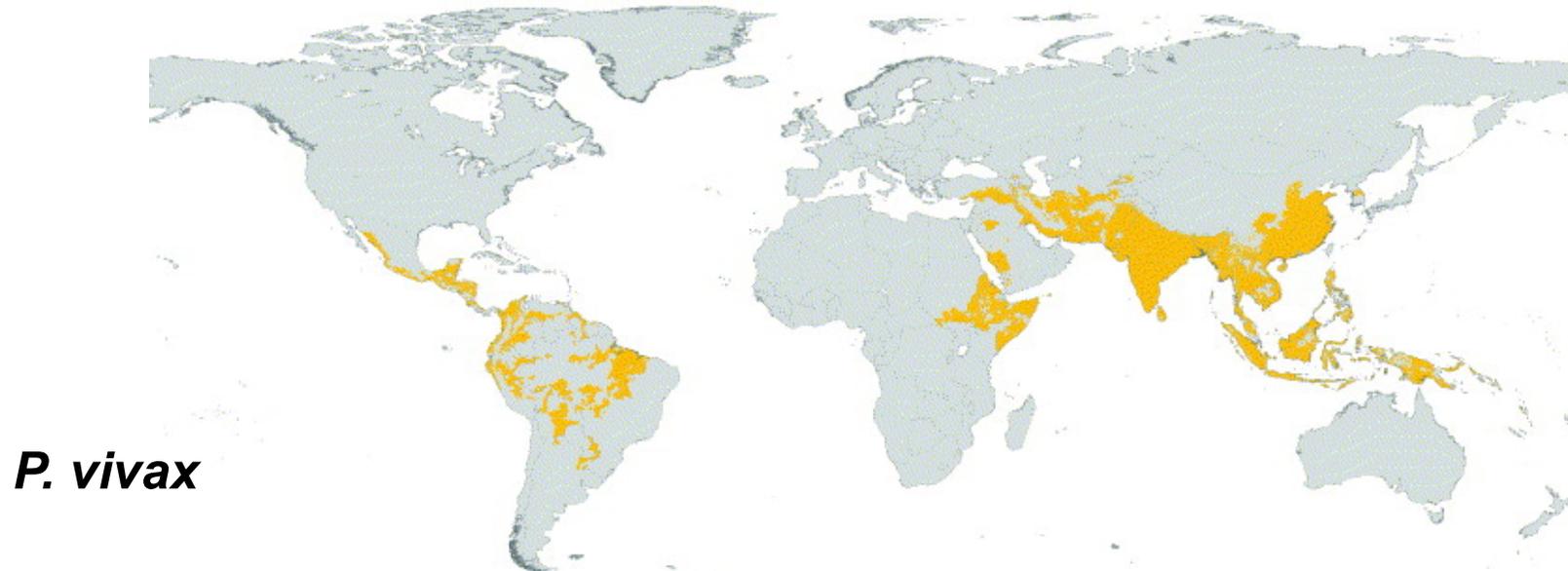


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Epidemiology of *P. vivax*

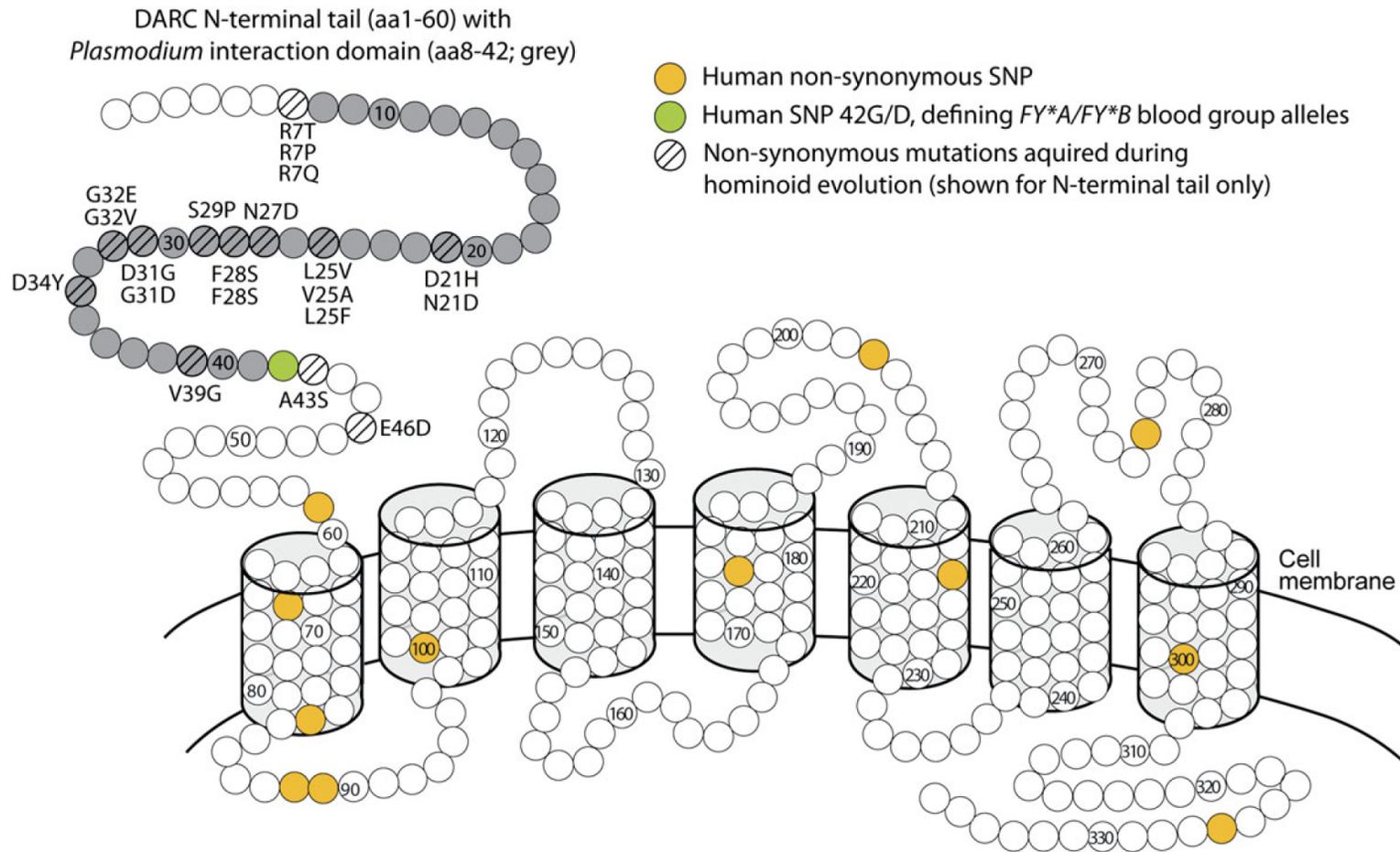


P. falciparum



P. vivax

Duffy Antigen Receptor for Chemokines (DARC)



chemokine receptor (Duffy antigen)
receptor for *P. vivax* and *P. knowlesi*

Duffy binding protein

Mutation in *cis* regulatory domain of DARC abrogates expression on erythrocytes

Distribution of **RBC Duffy Negativity** in Human Populations

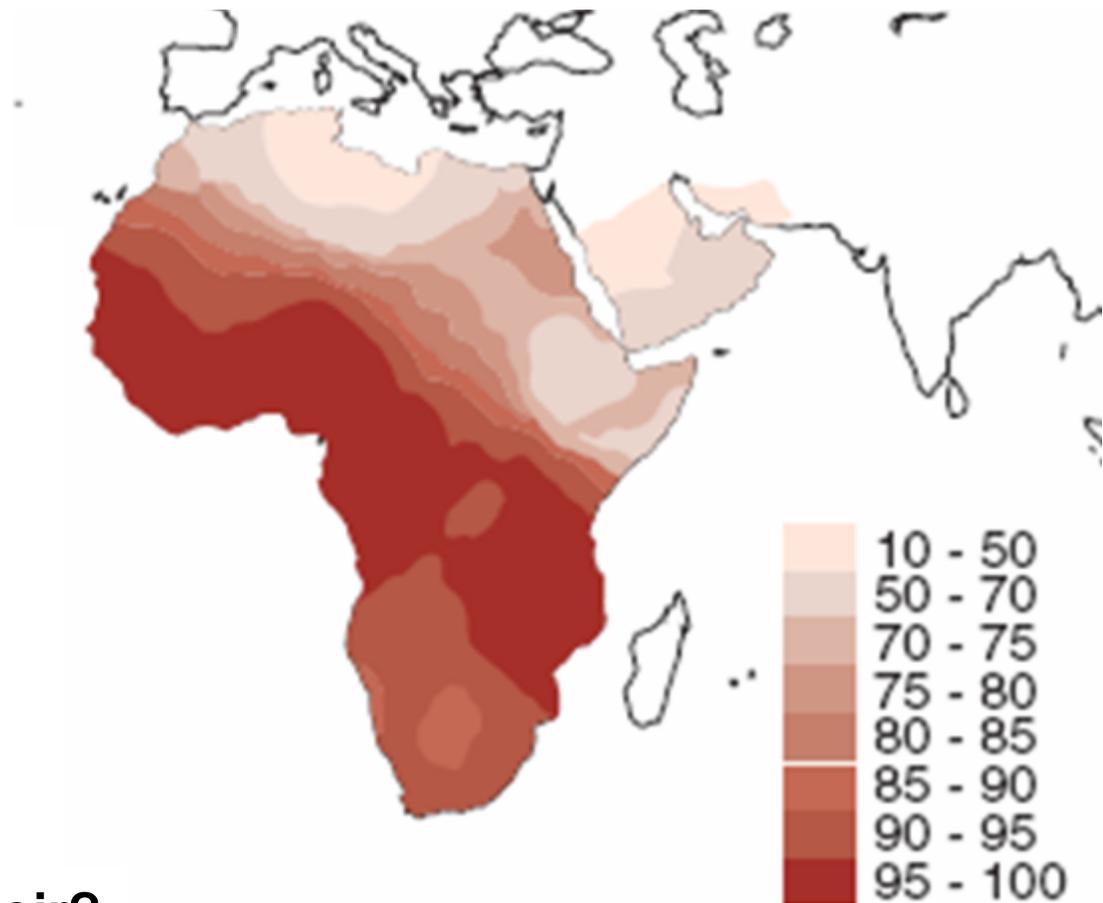
Homozygote RBC Duffy negative individuals are resistant to *P. vivax*

Human *P. vivax*

- absent from central and west Africa

~1% of travellers returning from central Africa with malaria have *P. vivax*
(Malaria Reference Laboratory, 1991-2001)

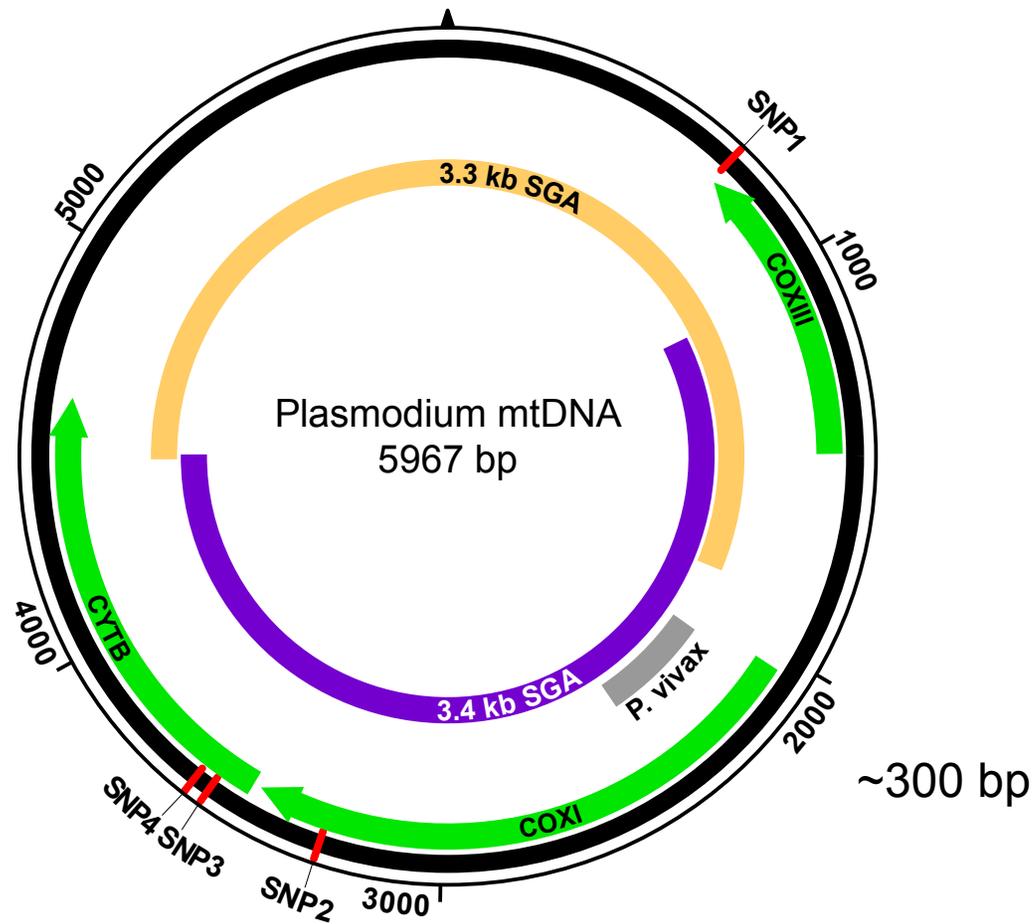
~13% of 400 humans in the Republic of Congo were reported to have antibodies to *P. vivax*
(Culleton et al., 2009)



Is there a natural reservoir?

Cavalli-Sforza et al. (1994) "The History and Geography of Human Genes"

Screening for *P. vivax* parasites in wild apes

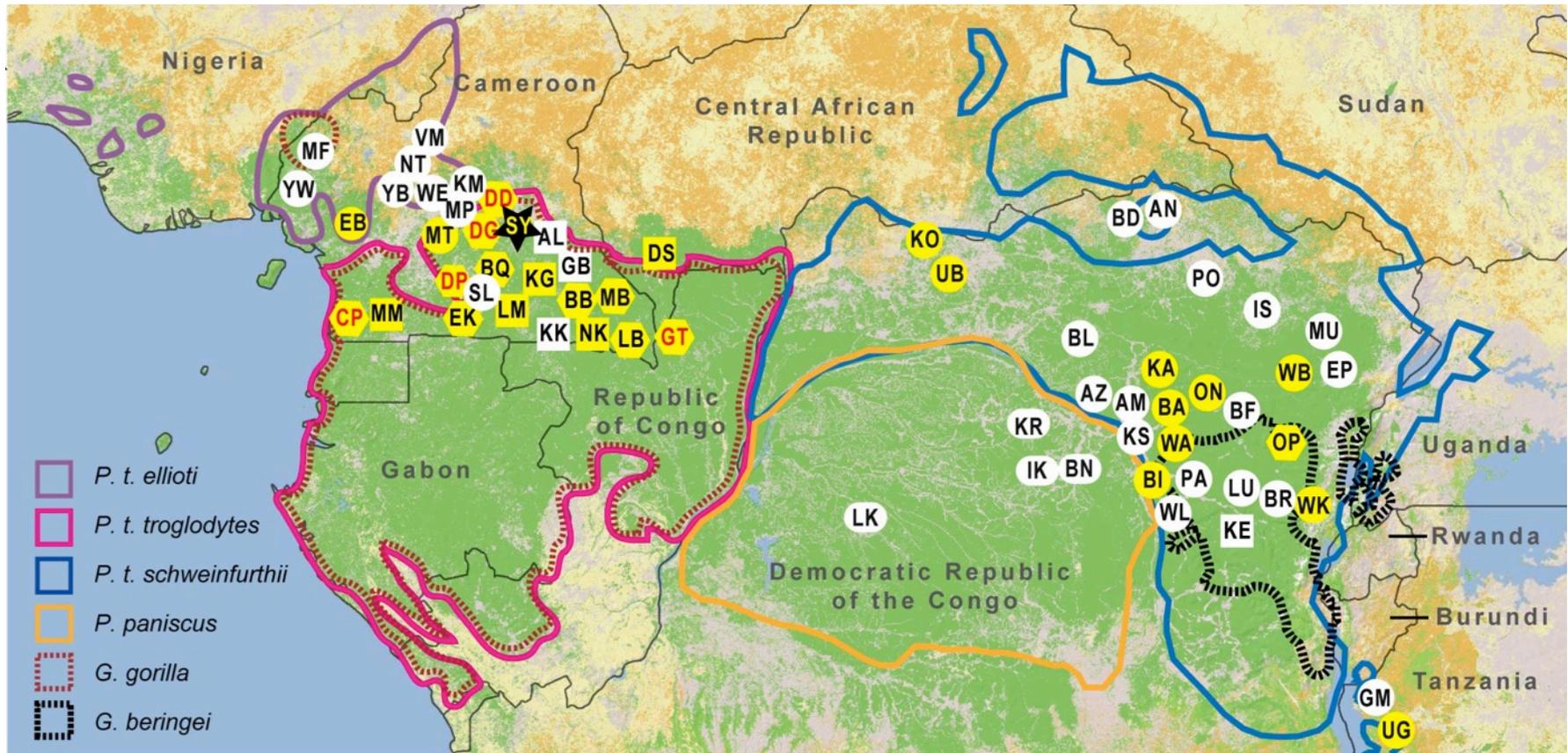


***P. vivax*-like Parasites in Wild-Living African Apes**

Species/ Subspecies	Field sites tested	Field sites positive	Fecal Samples tested	Fecal samples positive	Infection Rate (CI)
Nigeria Chimpanzee	14	0	126	0	0% (0-5%)
Central Chimpanzee	25	11	1,130	25	8% (6-10%)
Eastern Chimpanzee	28	10	1,615	20	4% (3-7%)
Western Gorilla	22	14	1,575	30	7% (5-9%)
Eastern Gorilla	4	1	189	2	4% (1-9%)
Bonobo	8	0	754	0	0% (0-1%)

Parasite rates of ape *P. vivax* are similar to those in endemically infected humans: Point estimates of patent blood infection rarely exceed 7%. Parasite rate of greater than 1% indicates stable transmission.

P. vivax-like Parasites in African Apes



Ape *P. vivax* is less commonly detected than *Laverania* species, but more widespread.

Genetic Characterization of Ape *P. vivax*

	Region	Length (bp)	Chimpanzees	Gorillas
mtDNA	diagnostic	297	54	33
	frag S	799	10	6
	frag A	1,922	7	6
	frag B	2,982	8	4
	frag C	2,134	5	2
Apicoplast DNA	frag D	2,536	15	10
	<i>clpC</i>	574	7	5
Nuclear DNA	<i>ldh</i>	724	5	2
	<i>asl</i>	838	4	1
	<i>crk2</i>	666	5	2
	<i>β-tub</i>	684	3	1



Plasmodium vivax in wild-living apes

mtDNA
frag D

P. vivax from:

central chimpanzees
Nigerian chimpanzees
eastern chimpanzees
western gorillas
humans

very closely related to human strains

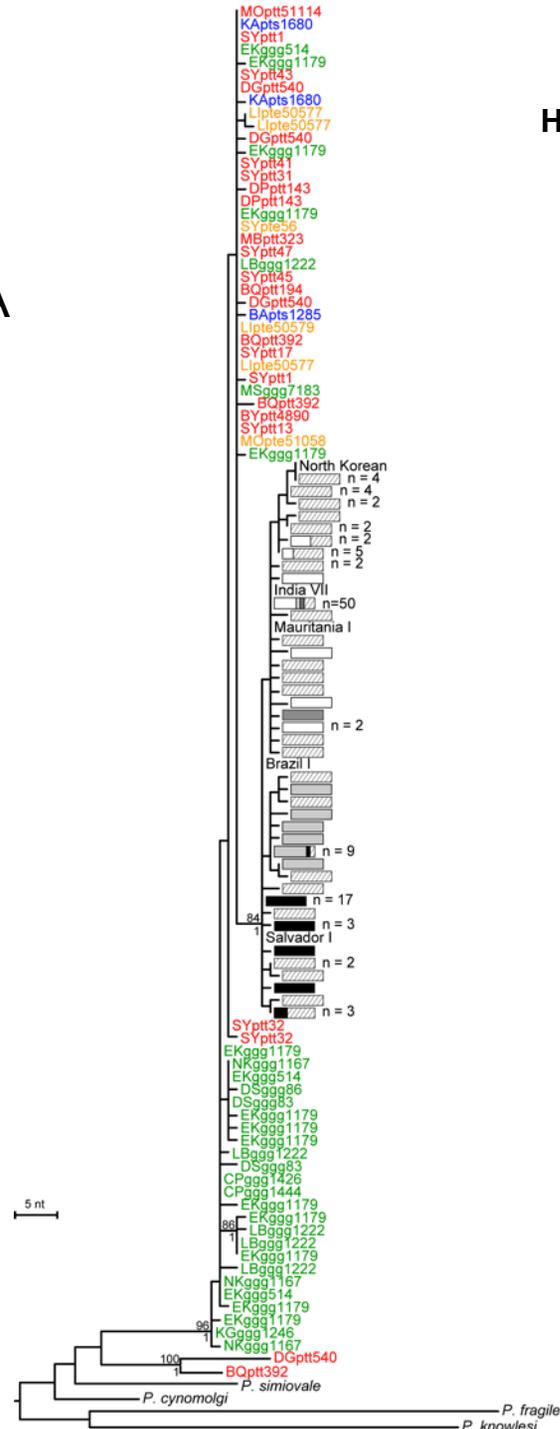
no species specificity among ape *P. vivax*

Human strains form monophyletic clade within the ape *P. vivax* radiation

Liu et al., *Nature Comm.*, 2014

Human *P. vivax* from:

Bangladesh,
Borneo,
Brazil,
China,
Colombia,
Dominican Republic,
El Salvador,
Ethiopia,
Honduras,
India,
Indonesia,
Iran,
Madagascar,
Melanesia,
Namibia,
Nicaragua,
Niger,
N. Korea,
Pakistan,
Panama,
Papua New Guinea,
Philippines,
Rwanda,
S. Korea,
São Tomé,
Solomon Islands,
Sri Lanka,
Tanzania,
Thailand,
Turkey,
Vanuatu,
Vietnam



Plasmodium vivax in wild-living apes

Nuclear DNA

ldh (700 bp)

samples from:

Humans

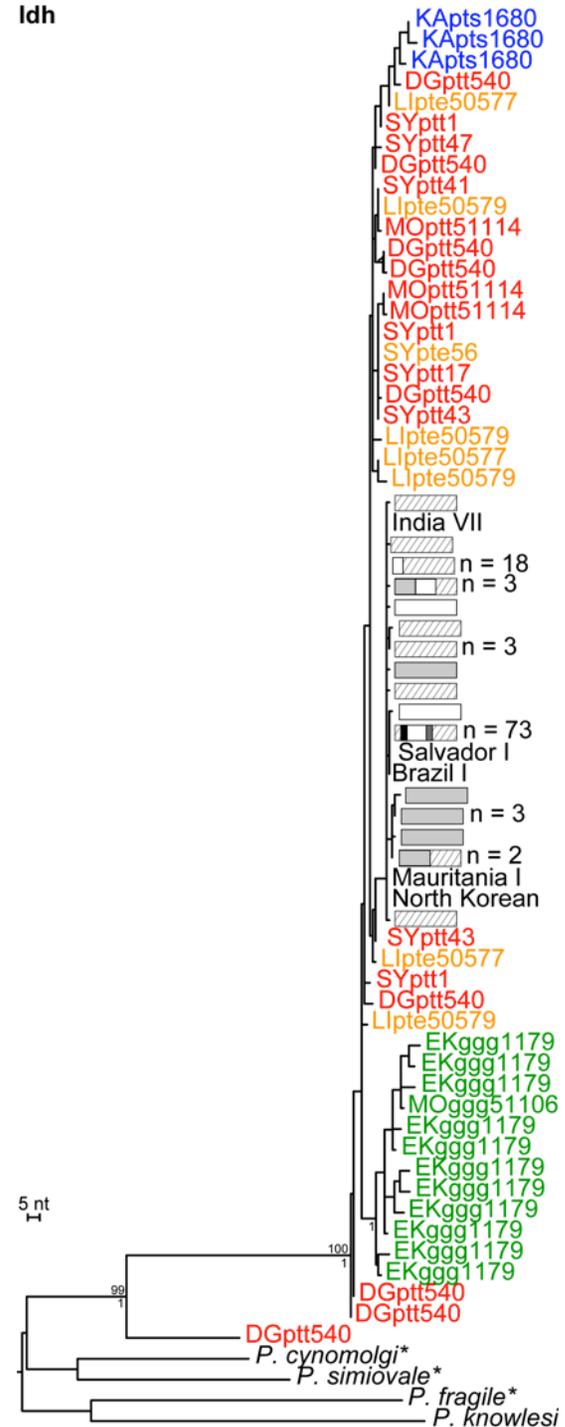
Nigerian chimpanzees

Central chimpanzees

Eastern chimpanzees

Gorillas

Liu et al., *Nature Comm.*, 2014



Plasmodium vivax in apes

Apicoplast DNA

clpC (570bp)

samples from:

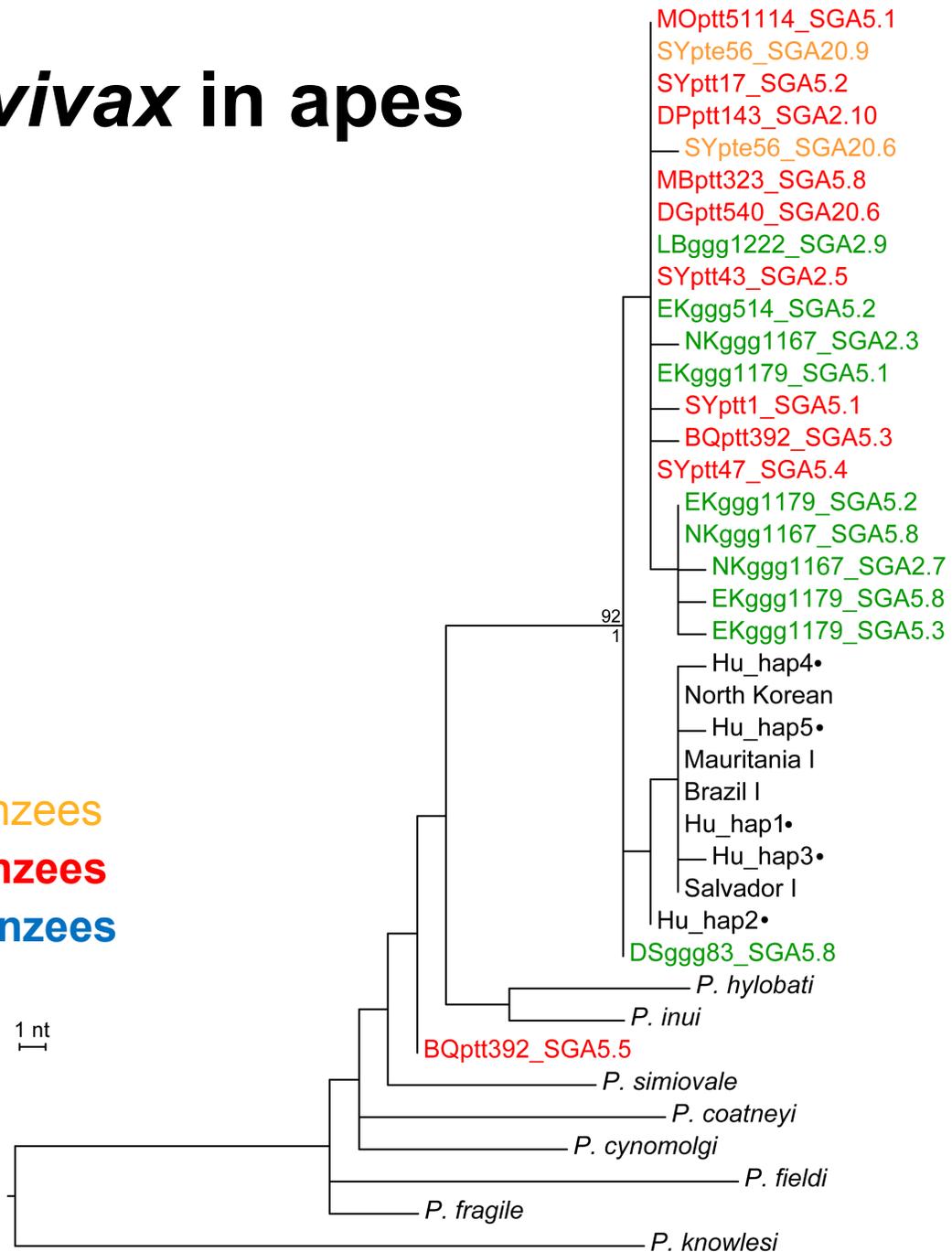
Humans

Nigerian chimpanzees

Central chimpanzees

Eastern chimpanzees

Gorillas

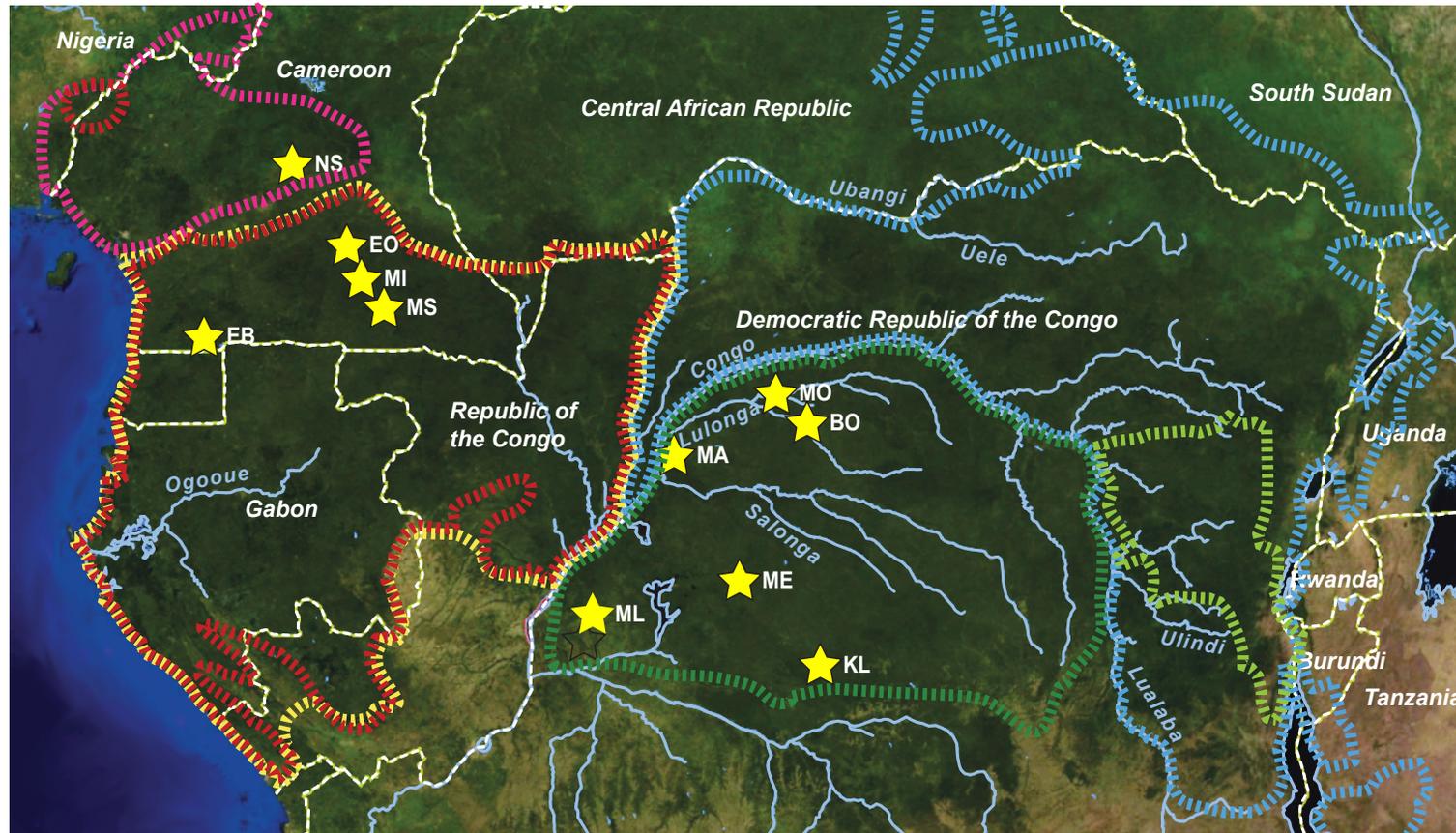


Sequence Diversity in Ape and Human *P. vivax*

<i>P. vivax</i> genome	Locus	Length (bp)	Human <i>P. vivax</i>			Ape <i>P. vivax</i>			π Ratio (A/H)
			No	π ($\times 10^{-3}$)	Ratio	No	π ($\times 10^{-3}$)	Ratio	
Mitochondrial	<i>cox1; cyt b</i>	2,443	138	0.75	1.0	62	1.02	1.0	1.4
Nuclear	<i>ldh</i>	679	114	1.58	2.1	42	14.07	13.8	8.9
Nuclear	<i>asl</i>	838	97	1.45	1.9	21	12.78	12.5	8.8
Nuclear	<i>crk2</i>	666	134	0.54	0.7	32	25.92	25.4	49.9
Nuclear	<i>β-tub</i>	684	81	0.81	1.1	12	11.85	11.6	14.6
Apicoplast	<i>clpC</i>	574	70	0.34	0.5	21	1.99	2.0	5.9

- Ape parasites were more diverse than human parasites at all loci tested.
- Ape *P. vivax* mtDNA sequences were 1.4 times more diverse than human sequences.
- Nuclear genes were 9 (*asl*, *ldh*) to 50 (*crk2*) times more diverse.
- Reduced diversity in human *P. vivax* reflects a recent bottleneck.

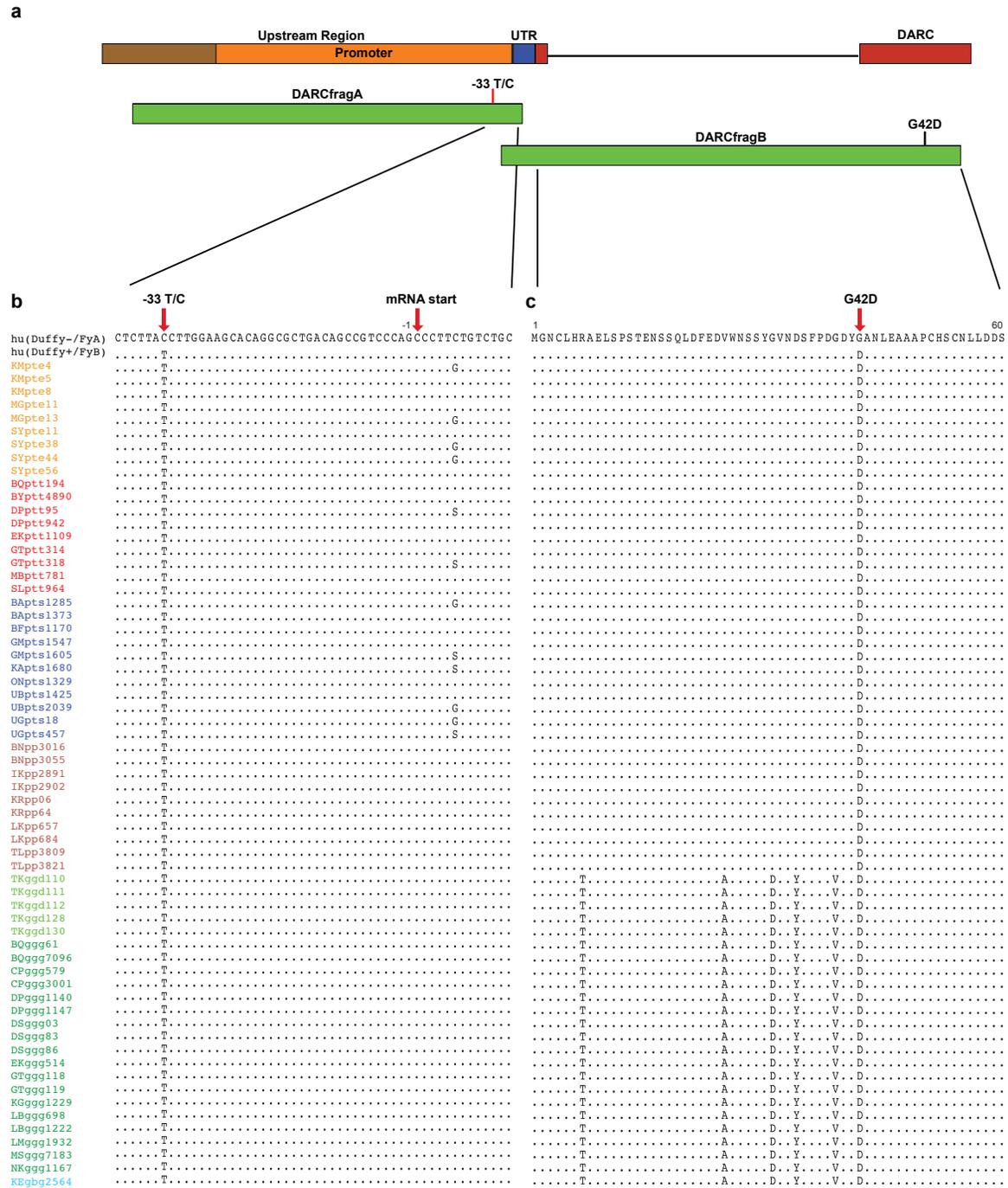
Failure to Detect *P. vivax* in African Monkey Species



- 998 blood samples primate bushmeat (16 primate species) at 11 different locations in Cameroon and DRC.
- No evidence for *P. vivax*-like parasites
- 501 of 998 samples (50.2%) were positive for *Hepatocystis* spp.

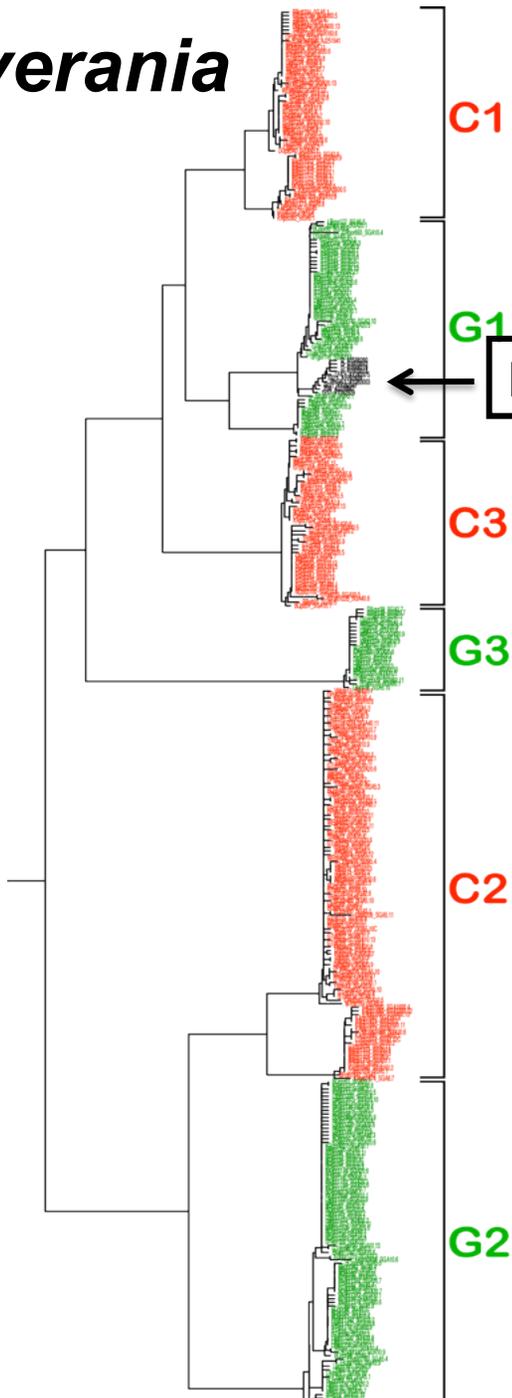
DARC promoter in apes

- 134 ape samples
- 28 bonobos
- no evidence for a Duffy negative phenotype (C at position -33) or the selection of a potentially protective allele (FyA)



Laverania

P. vivax



C1

G1

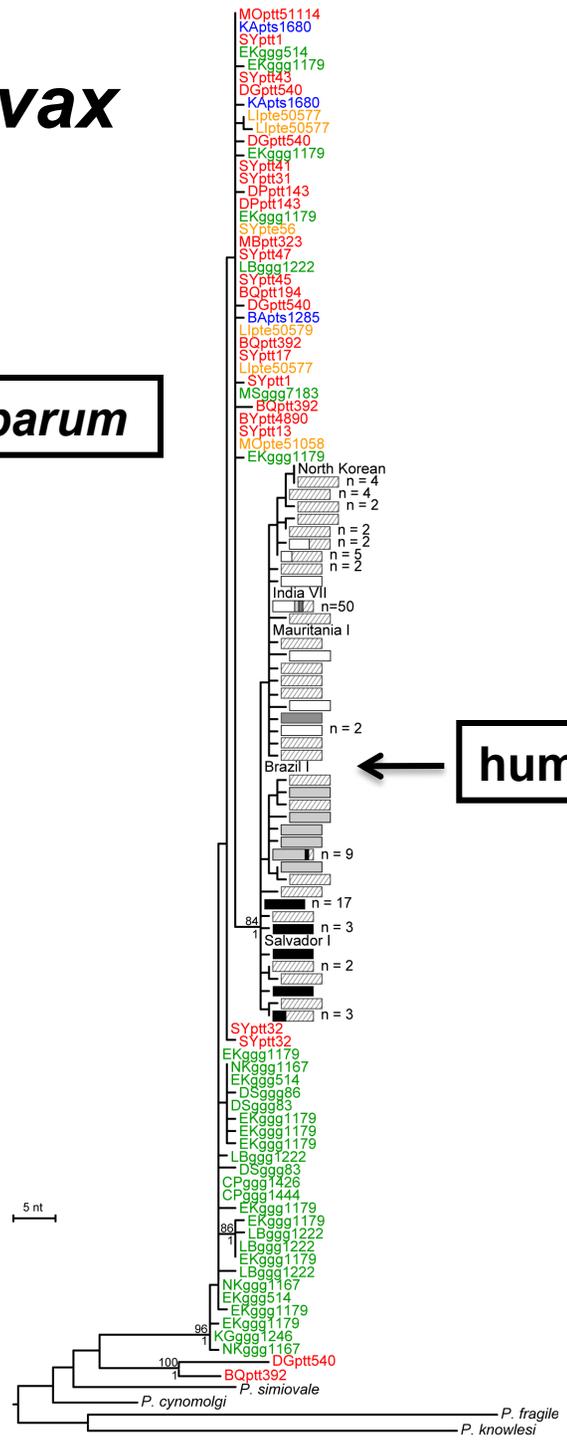
C3

G3

C2

G2

human *P. falciparum*



MOpt51114
KApts1680
SYptt1
EKggg514
EKggg1179
SYptt43
DGApt540
KApts1680
Lpte0577
Lpte50577
DGApt540
EKggg1179
SYptt44
SYptt31
DPtt143
DPtt143
EKggg1179
SYptt56
MBpt323
SYptt47
LBggg1222
SYptt45
BOpt194
DGApt540
BApts1285
Lpte50579
BOpt392
SYptt17
Lpte50577
SYptt1
MSggg7183
BOpt392
BYpt4890
SYptt13
MOpt51058
EKggg1179

North Korean
n = 4
n = 2
n = 2
n = 2
n = 5
n = 2
India VII
n = 50
Mauritania I
n = 2
Brazil I
n = 9
n = 17
n = 3
Salvador I
n = 2
n = 3

SYptt32
SYptt33
EKggg1179
NKggg1167
EKggg514
DSggg86
DSggg83
EKggg1179
EKggg1179
Lpte50577
LBggg1222
DSggg83
CPggg1426
CPggg1444
EKggg1179
EKggg1179
LBggg1222
LBggg1222
EKggg1179
LBggg1222
NKggg1167
EKggg514
EKggg1179
EKggg1179
KGggg1246
NKggg1167
DGApt540
BOpt392
P. cynomolgi
P. simiovale
P. fragile
P. knowlesi

5 nt

human *P. vivax*

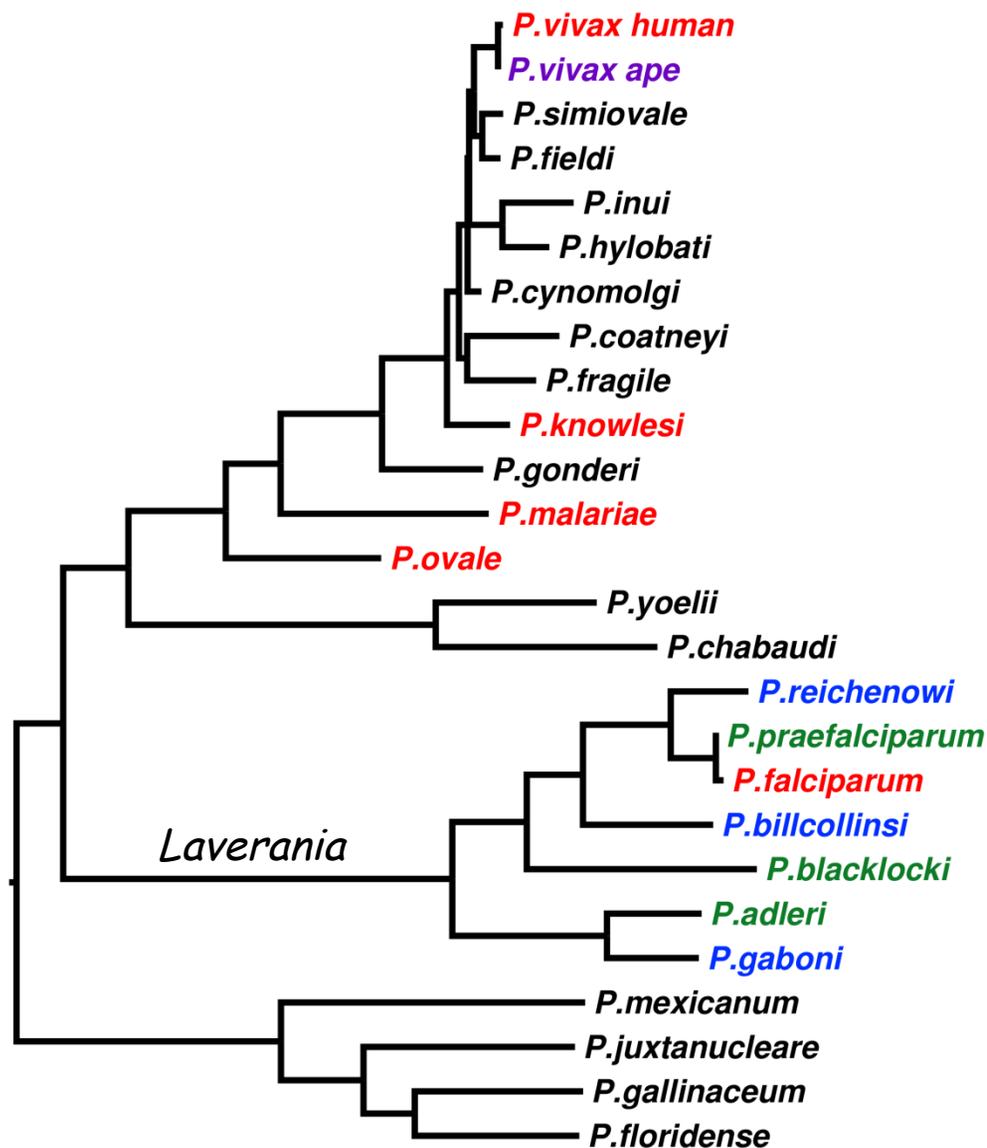
Origin of human *P. vivax*

- *P. vivax* does not divide into chimpanzee and gorilla specific lineages.
- Humans, gorillas and chimpanzees in Africa were likely infected with an ancestral stock of *P. vivax* until the Duffy negative mutation started to spread and eliminated *P. vivax* from humans there.
- Thus, extant human *P. vivax* represents a bottlenecked lineage that survived after spreading out of Africa.

Take Home Points

- Chimpanzees and gorillas (but not bonobos) in central Africa represent a substantial *P. vivax* reservoir.
- There is no evidence of *P. vivax*-like infections in other African primates.
- There is no evidence for a Duffy negative phenotype or the selection of a protective allele in wild apes.
- Human *P. vivax* arose from within a *Plasmodium* species that infects chimpanzees and gorillas.
- This provides a long-sought explanation for the selection of the Duffy negative phenotype in west central Africa.

African Great Apes are Infected with a Plethora of *Plasmodium* Species

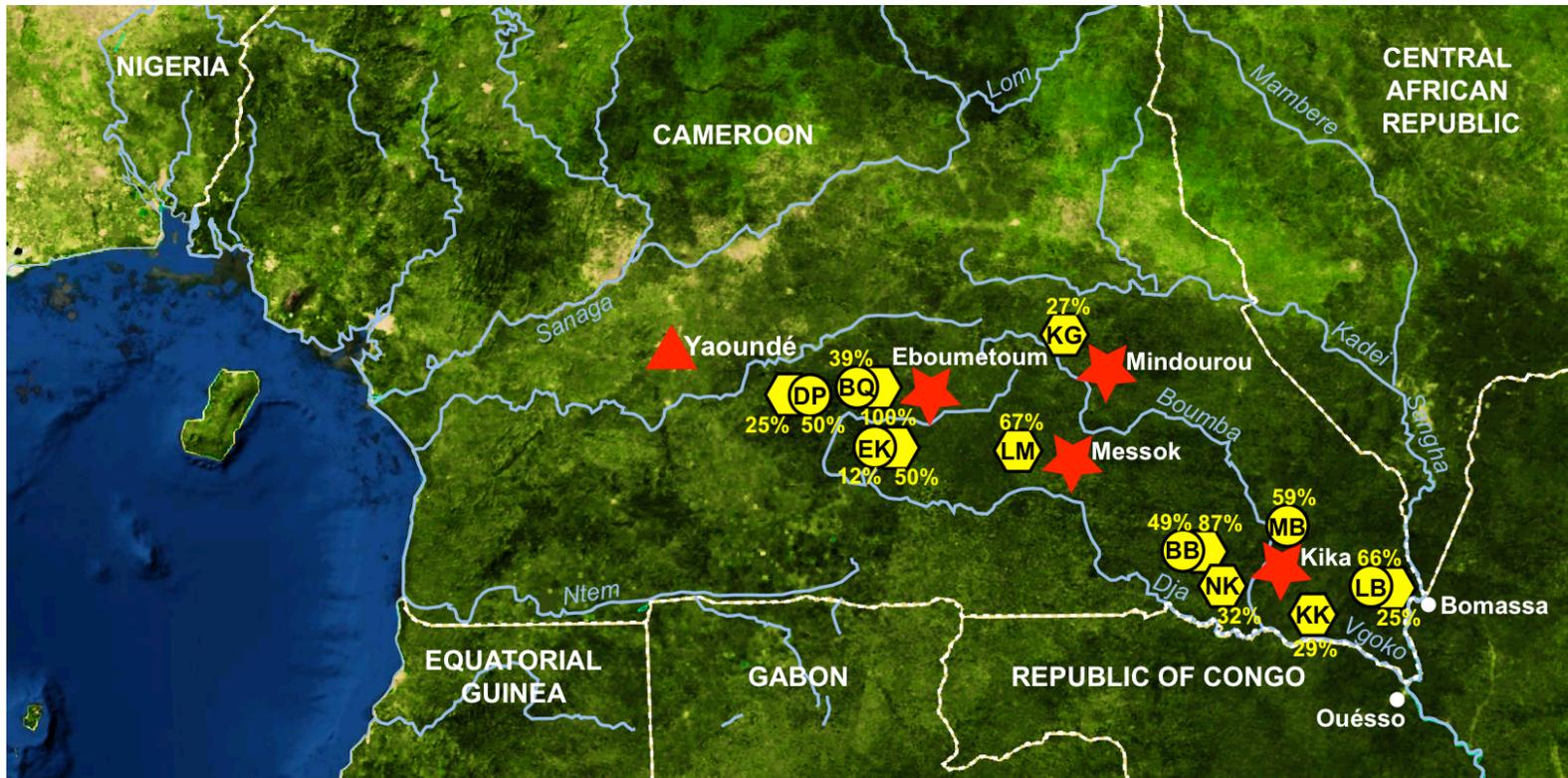


Does cross-species transmission occur in areas where infected apes and humans live in close proximity?



Sesh Sundararaman

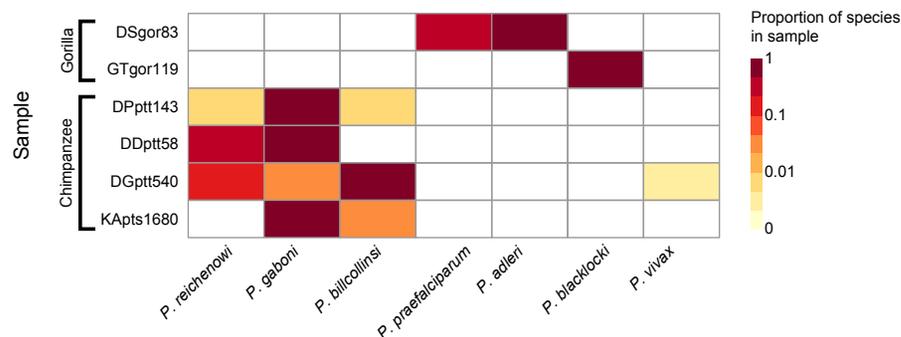
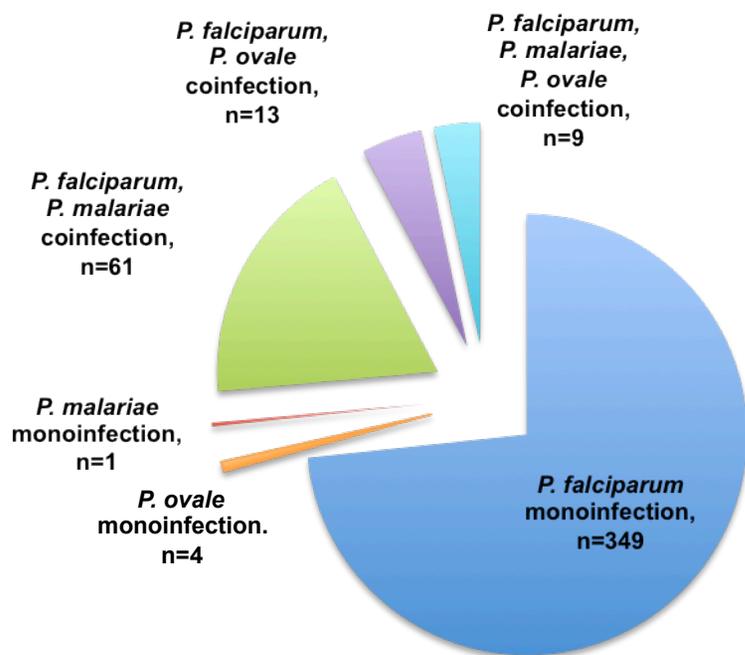
Screening Individuals Native to Southern Cameroon for Ape *Plasmodium* Infections



- 1,403 human buffy coat samples from Southern/Central Cameroon
 - forest dwellers and hunters from eight villages
 - all living in close proximity to infected wild apes



Plasmodium Pyrosequencing Results

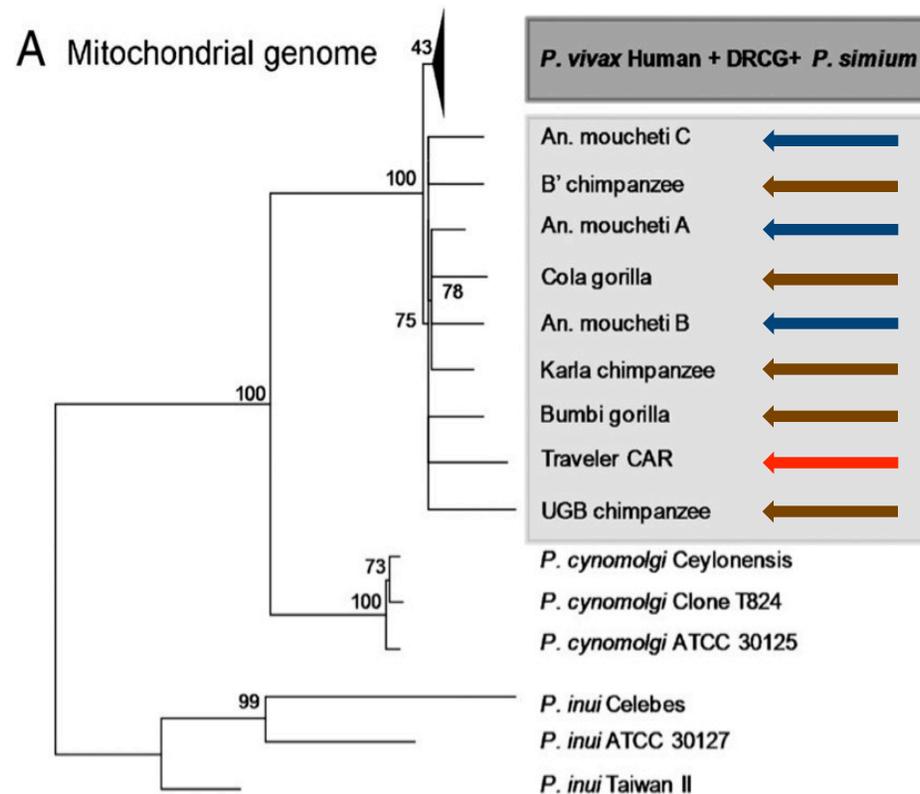


Infection Status of 437 Barcoded Human Samples

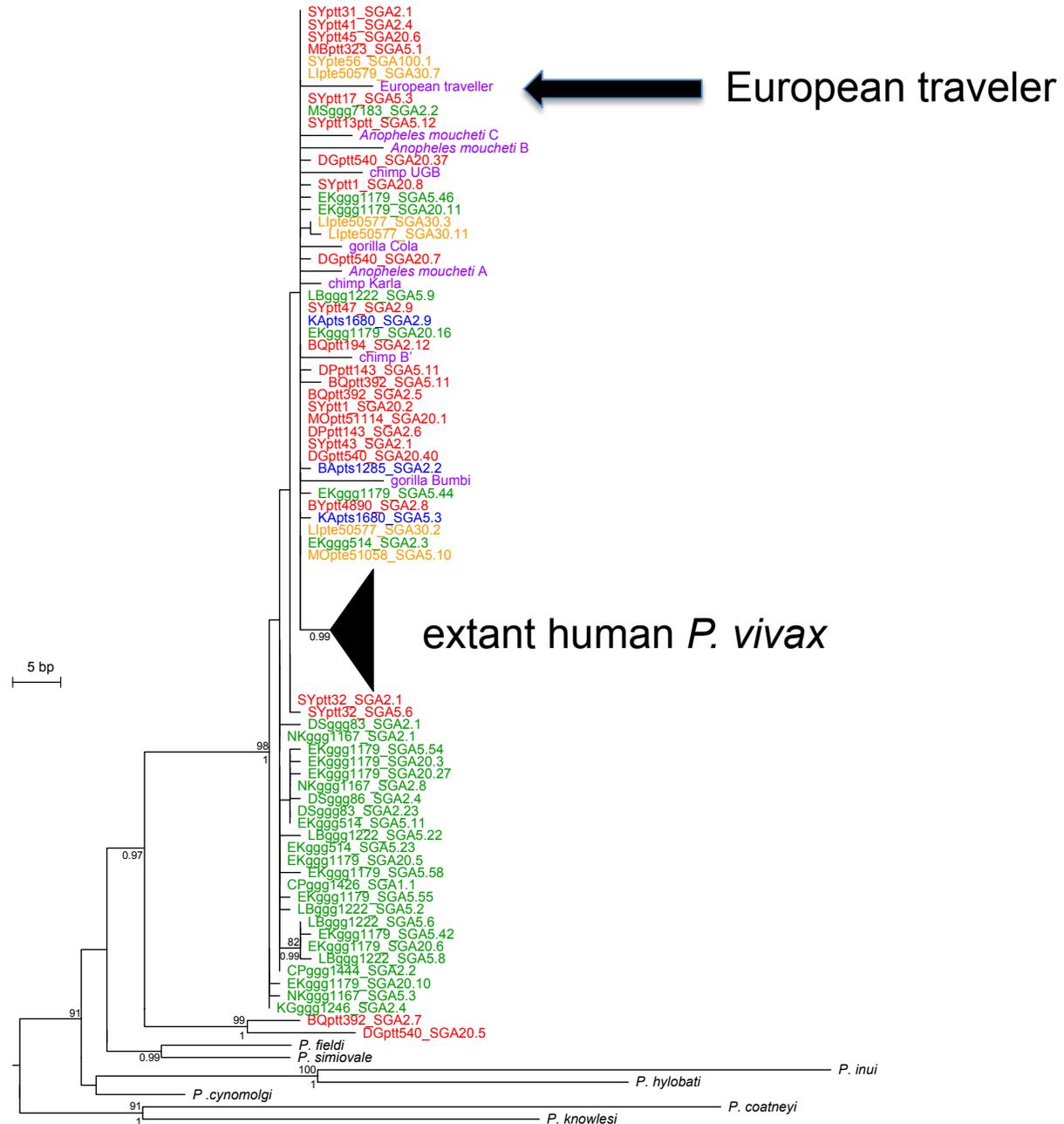
No evidence of ape *Laverania* species or *P. vivax* in human samples from Cameroon

Diversity, host switching and evolution of *Plasmodium vivax* infecting African great apes

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Ape *P. vivax* Infects Humans



Transmission Experiments Involving Apes and Humans

P. vivax and P. ovale: “*P. schwetzi*” was transmitted from apes to humans on more than one occasion. At least once, “*P. schwetzi*” may have been a *P. vivax*-related parasite because transmissions were successful only when the recipients were Caucasians, but not if they were Africans.

P. falciparum: All attempts to transmit “*P. reichenowi*” from chimpanzees to humans failed.

Take Home Points

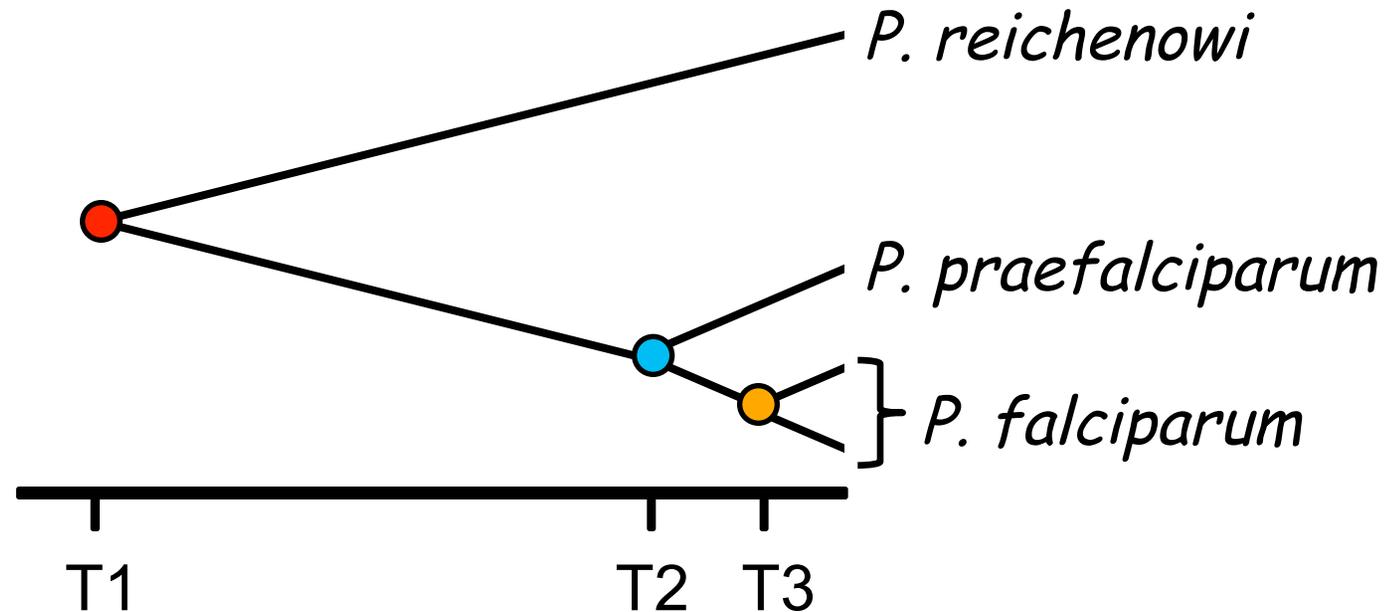
- Wild apes infected with *Laverania* parasites do not serve as a recurring source of human malaria.
- What circumstances led to the emergence of *P. falciparum* in humans?
- Humans are susceptible to infection with ape *P. vivax*.
- What would happen if ape *P. vivax* were exported to areas where human *P. vivax* is actively transmitted?

How old is human malaria?

Ancient: hundreds of thousands of years old
based on molecular clocks
requires assumption that parasites have
co-evolved with their host
rates of rRNA evolution (bacteria)

Recent: <10,000 years old
based on parasite biology
genetic variants are young:
HbC has not yet spread to fixation
G6PD <10,000 years old
Duffy emerged ~30,000 years ago
transmission dynamics
domestication of *Anopheles gambiae*

Timescale of *Plasmodium* in humans?



T1/T3 = 25 (mtDNA; HUGHES & VERRA 2010)
= 20 (nDNA; SILVA *et al.* 2011)

If T1 = 6 Myr ago, T3 = 240-300 kyr ago

Mutation rate of *Plasmodium falciparum*

MOSQUITO: 14 genome replications in 19 days

LIVER: 15 genome replications in 5 days

BLOOD: 95 genome replications in 66 days

TRANSMISSION CYCLE: 124 replications in 90 days
= 500 replications per year (range: 270-600)

MUTATION RATE:

2.1×10^{-9} mutations per site per replication

PAGET-McNICOL & SAUL (2001) *Parasitology* 122:497

$1.0-9.7 \times 10^{-9}$ mutations per site per replication

BOPP *et al.* (2013) *PLoS Genetics* 9:e1003293

Assuming 300 replications per year and a mutation rate of 1×10^{-9} mutations per site per replication, the expected neutral evolution rate is 3×10^{-7} substitutions per site per year. At this rate, divergence of 2.9×10^{-3} substitutions per site (divergence at synonymous sites) would reflect a common ancestor about 4800 years ago.

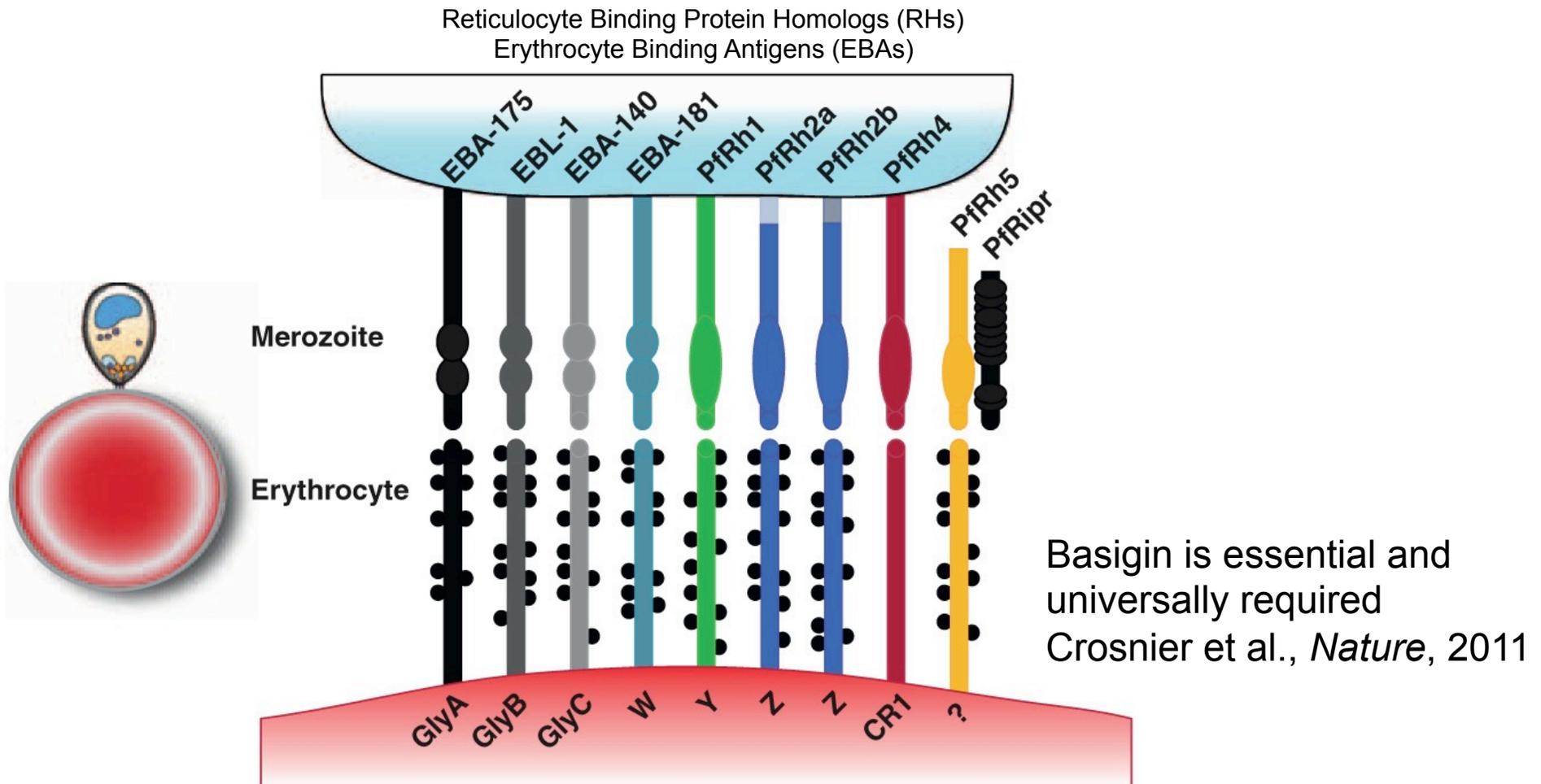
Take Home Points

- The diversity among extant *P. falciparum* strains can easily be explained by descent from an ancestor that existed within the last 10,000 years.
- *P. vivax* is more diverse than *P. falciparum*.
- Because of the hypnozoite stage, the number of genome replications is more difficult to estimate.
- The age of the Duffy mutation (10,000 – 30,000 years) is likely a better calibration point.
- Using mutation rates seem more credible than molecular clock estimates which are based on unsubstantiated assumptions.

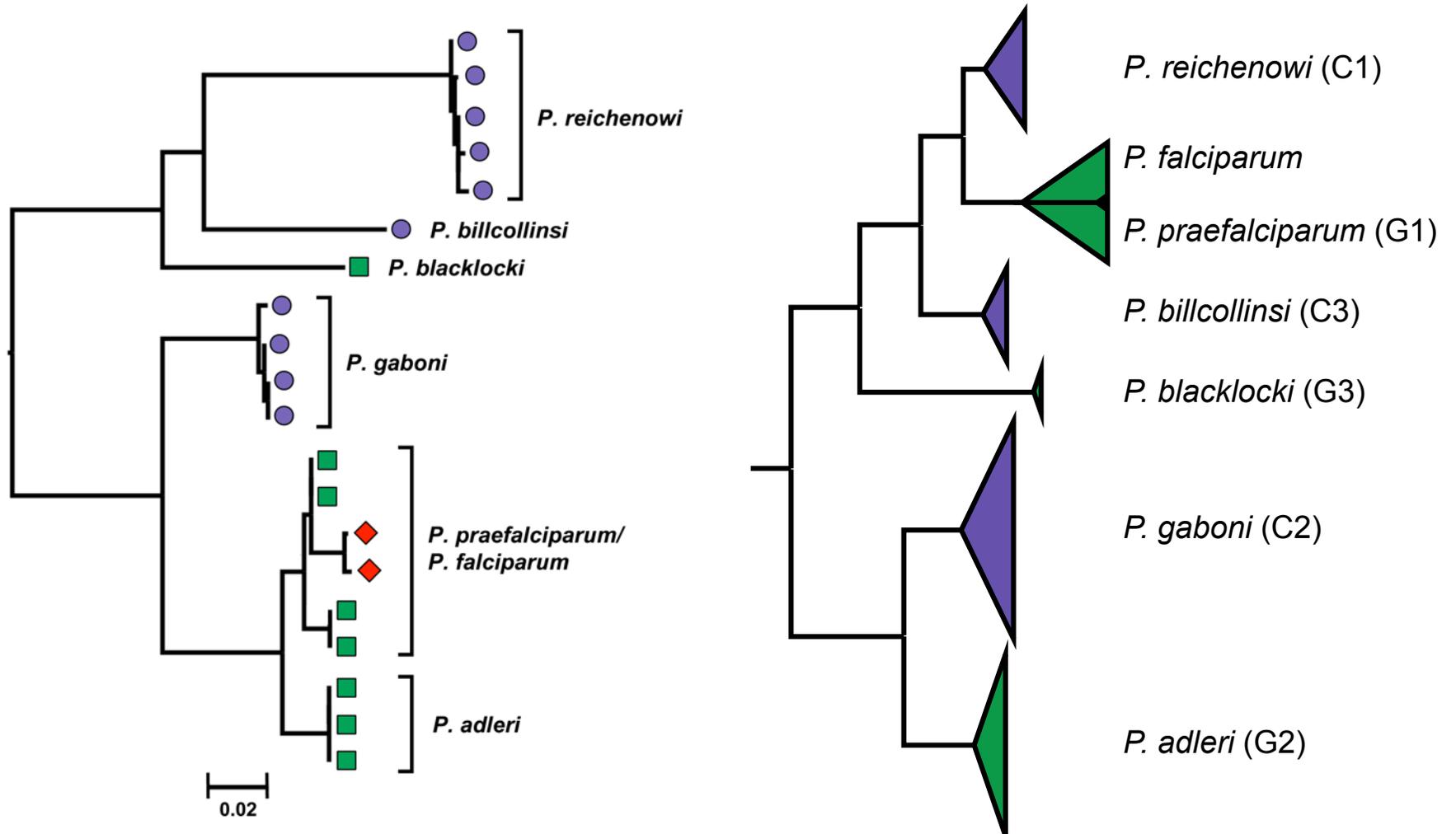
Future Studies

- Ape *Laverania* parasites are highly species specific.
- How did the gorilla precursor of *P. falciparum* manage to overcome this barrier?

Plasmodium/Host Interactions



Horizontal gene transfer in the RH5 locus of *P. praefalciparum*

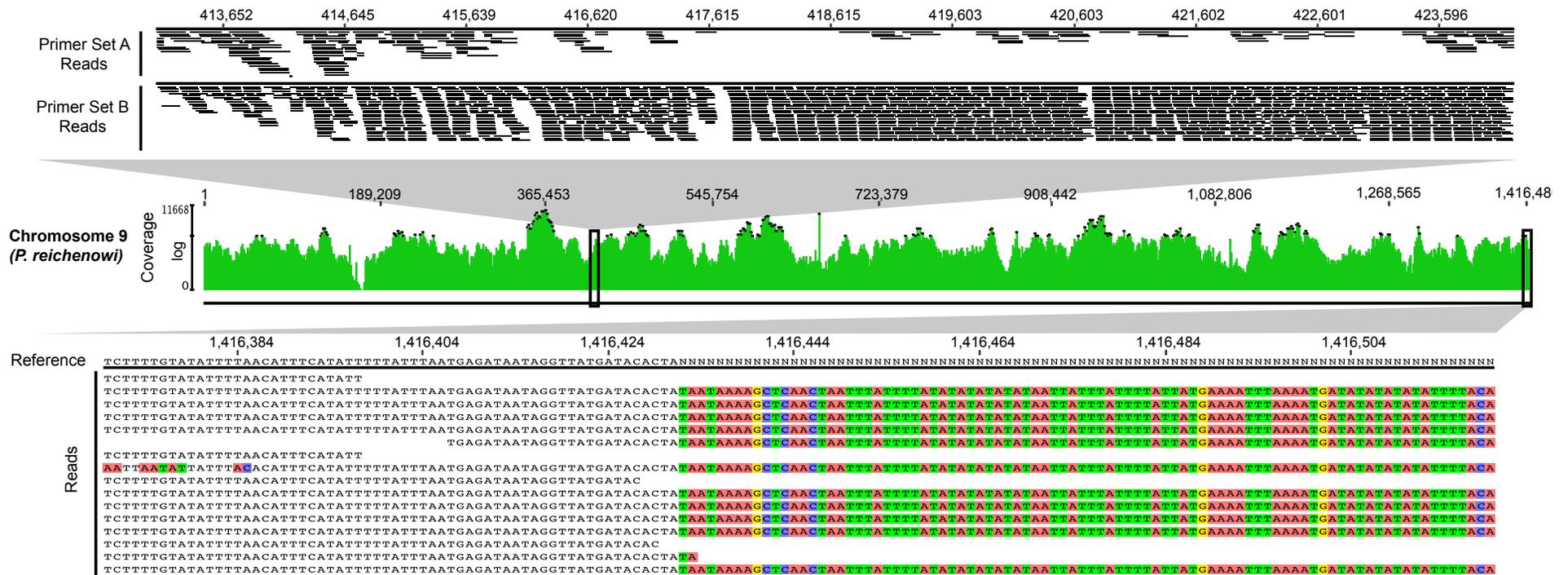


need comparative genomic studies

Selective Whole Genome Amplification

- Each species has unique mutational biases.
- Identify sequence motifs that are over-represented in *Plasmodium* genomes, but under-represented in contaminating DNA.
- Design *Plasmodium* specific SWGA primers.
- Amplify ape *Plasmodium* genomes after digestion with methylation dependent restriction enzymes.
- >30,000-fold enrichment.

Selective Whole Genome Amplification of *P. reichenowi* from Unfractionated Chimpanzee Blood



Conclusions

- Three major human pathogens originated in apes.
- The transmission hurdle for ape pathogens is lower; still, only a small subset has successfully colonized humans.
- Non-invasive studies of wild apes have been key.
- There is value studying the ape precursors of human pathogens in their natural hosts.

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